Impact factor 5·9

Eurosurveillance

Europe's journal on infectious disease epidemiology, prevention and control

Vol. 22 | Weekly issue 23 | 08 June 2017

ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

www.eurosurveillance.org

Whole genome sequencing (WGS) for food-borne pathogen surveillance and control – taking the pulse

J Moran-Gilad [1 2 3]
1. Ministry of Health, Public Health Services, Israel
2. Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel
3. ESCMID Study Group for Genomic and Molecular Diagnostics, Basel, Switzerland

Correspondence: Jacob Moran-Gilad (giladko@post.bgu.ac.il)

Citation style for this article:
Moran-Gilad J. Whole genome sequencing (WGS) for food-borne pathogen surveillance and control – taking the pulse. Euro Surveill. 2017;22(23):pii=30547. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2017.22.23.30547

Article submitted on 02 June 2017 / accepted on 08 June 2017 / published on 08 June 2017

Next-generation sequencing (NGS) is transforming microbiology [1]. With the increased accessibility and decrease in the costs of sequencing and the optimisation of the 'wet laboratory' components of NGS i.e. the quality and throughput of DNA extraction, library preparation and sequencing reactions, whole genome sequencing (WGS) of bacterial isolates is rapidly revolutionising clinical and public health microbiology. WGS is a 'disruptive technology' that has the potential to become a one-stop-shop for routine bacterial analysis. By replacing multiple parallel steps in the microbiology diagnostic cycle, which currently involves traditional and molecular methods, it achieves accurate and speedy species identification, inference of antimicrobial susceptibility and virulence and high-resolution subtyping [2].

Typing of food-borne pathogens was one of the earliest applications of WGS [3] and proof-of-concept has been demonstrated for the superiority of WGS over traditional typing methods such as pulsed-field gel electrophoresis (PFGE), multilocus variable-number tandem repeat analysis (MLVA) and multilocus sequence typing (MLST), for a range of high priority food-borne pathogens, including *Salmonella enterica, Listeria monocytogenes, Campylobacter* species and Shiga-toxin producing *Escherichia coli* [4]. Applications of WGS include the investigation of food-related outbreaks and surveillance to delineate the local, regional and global genomic epidemiology of pathogens and to attribute the infection source. WGS thus supports risk assessment and guides interventions for prevention and control of infections.

A growing number of (public health microbiology) laboratories and governmental agencies employ WGS in their routine practice and food-borne pathogen surveillance and even more are expected to enter this field in the near future. Thus the maturation of food-borne pathogen surveillance into the WGS era is very timely.

In order for WGS to be adopted as the new gold standard for tracking of food-borne pathogens, a key element of food-borne disease control, there is a need for robust, standardised, portable and scalable methods for analysing WGS data. However, the notable diversity of bioinformatics tools and approaches used for bacterial WGS to date, as evident from a recent survey by the Global Microbial Identifier project [5], creates a tremendous challenge for harmonising surveillance and investigation of food-borne illness, especially across geographical borders and different sectors. Calling variants based on analysis of single nt polymorphisms (SNPs) as it is being done in many food-borne outbreak investigations, offers maximal resolution and discriminatory power but is very difficult to standardise. Therefore, approaches based on gene-by-gene analyses, collectively referred to as 'extended MLST', such as core genome (cg) or whole genome (wg)MLST may be advantageous [6], and have been advocated in other public health settings, such as Legionnaires' disease control [7].

PulseNet was established in the United States (US) more than 20 years ago as a laboratory network for molecular epidemiology based on standardised PFGE analysis and later expanded globally. PulseNet has been successful in engaging many players in the field of food safety on a global scale and in creating a platform for data sharing and comparison of clinical, veterinary and food isolates in over 80 countries and it has a proven track-record in supporting molecular surveillance [8]. Nevertheless, some issues remained unresolved such as creation and implementation of a global nomenclature, which is important for communicating molecular epidemiology results, both scientifically as well as operationally.

In this issue of *Eurosurveillance*, an article by Nadon et al. [9] describes the next generation of PulseNet International, which is evolving into harnessing WGS.

This initiative represents a wide collaboration between many leading agencies and stakeholders in this area, including the US Centers for Disease Control and Prevention (CDC), the European Centre for Disease Prevention and Control (ECDC) and the Public Health Agency Canada (PHAC), just to name a few. The authors illustrate the technical and practical aspects of adapting the network. Notably, PulseNet International has chosen an extended MLST approach, specifically, wgMLST, as its default phylogenetic analysis tool, which should underpin a standardised and efficient nomenclature-based system. Different technical and practical aspects are reviewed and discussed, mainly focusing on information technology (IT) and bioinformatics aspects (data storage, computing power, nomenclature, data sharing), methods for validation and quality control/quality assurance. Nadon et al. highlight complexities surrounding the implementation of WGS for food-borne disease surveillance, with respect to readiness at individual country and regional levels and delineate how PulseNet plans to address these.

The evolution of PulseNet International is very encouraging and will reinforce the use of NGS in the area of food safety. That said, challenges remain that need to be addressed by the public health community. There is a need for user-friendly bioinformatics solutions that will enable automated analysis of bacterial genomes by non-experts in bioinformatics to extract valuable information in a time-efficient manner. Such solutions should offer as much backwards compatibility as possible with current typing methods since the global transition to WGS is expected to be gradual. It should also offer an efficient strain/allele nomenclature that facilitates inter-laboratory work. Moreover, bioinformatics solutions should also factor in the developments in the field of DNA sequencing, particularly long-read single molecule sequencing platforms and portable sequencing devices which are increasingly being used. While WGS of food-borne pathogens has now become the new gold standard for food-borne pathogen typing, other techniques such as strain typing and characterisation using proteomics (particularly matrix-assisted laser desorption/ionisation (MALDI) time-of-flight (TOF) mass spectroscopy) or DNA arrays are rapidly evolving and should be carefully evaluated [10]. The field of metagenomics is also rapidly advancing and culture-independent microbiology, enabling genomic analysis of pathogens directly from sequenced clinical or environmental samples (as opposed to cultured isolates), is just around the corner [11]. When laying the foundations for global food pathogen surveillance networks for the coming years, we need to be mindful of such future developments.

Different from current protocols in which only typing results are shared, the transition to genome-based surveillance inevitably involves the sharing of complete sequence data. This has many implications, not only with respect to data storage, analysis and sharing infrastructures, but also aspects such as data ownership, privacy and transparency, pertaining to both genomic sequences and the related metadata. These issues should be proactively addressed in order to provide reassurance concerning data protection and create flexible solutions that will facilitate the timely sharing of public health data by as many partners as possible.

Finally, the transition to WGS-based surveillance needs to ensure sufficient quality is maintained in order to meet national and international regulatory requirements. Nadon et al. rightfully emphasise in their paper, the importance of validation, quality control and standardisation. One major aspect in making this transition and that needs to be considered is the human factor. The successful implementation of WGS-based surveillance on a global scale requires careful planning, building of capacity and training of public health and microbiology personnel to develop local readiness, especially in limited resource settings. Care should be taken to address the 'softer' issues, including possible cultural, political and cross-sector barriers, which together with economical, management and operational aspects could greatly influence the successful implementation of WGS.

This is a fascinating time for public health microbiology, and initiatives such as the integration of WGS as proposed by PulseNet International, are central for leveraging recent technological advancements for the benefit of public health surveillance.

Conflict of interest

None declared.

References

1. Didelot X, Bowden R, Wilson DJ, Peto TE, Crook DW. Transforming clinical microbiology with bacterial genome sequencing.Nat Rev Genet. 2012;13(9):601-12. DOI: 10.1038/nrg3226 PMID: 22868263
2. Deurenberg RH, Bathoorn E, Chlebowicz MA, Couto N, Ferdous M, García-Cobos S, et al. Application of next generation sequencing in clinical microbiology and infection prevention. J Biotechnol. 2017;243:16-24. DOI: 10.1016/j.jbiotec.2016.12.022 PMID: 28042011
3. The INFOSAN Activity Report 2014/2015 [Internet]. Geneva: World Health Organization; Geneva. Available from: http://www.who.int/foodsafety/publications/infosan_activity2014-15/en/
4. Ronholm J, Nasheri N, Petronella N, Pagotto F. Navigating microbiological food safety in the era of whole-genome sequencing.Clin Microbiol Rev. 2016;29(4):837-57. DOI: 10.1128/CMR.00056-16 PMID: 27559074
5. Moran-Gilad J, Sintchenko V, Pedersen SK, Wolfgang WJ, Pettengill J, Strain E, et al. Proficiency testing for bacterial whole genome sequencing: an end-user survey of current capabilities, requirements and priorities. BMC Infect Dis. 2015;15(1):174. DOI: 10.1186/s12879-015-0902-3 PMID: 25887164
6. Maiden MC, Jansen van Rensburg MJ, Bray JE, Earle SG, Ford SA, Jolley KA, et al. MLST revisited: the gene-by-gene approach to bacterial genomics. Nat Rev Microbiol. 2013;11(10):728-36. DOI: 10.1038/nrmicro3093 PMID: 23979428
7. Moran-Gilad J, Prior K, Yakunin E, Harrison TG, Underwood A, Lazarovitch T, et al. Design and Application of a Core Genome Multi-Locus Sequence Typing Scheme for Investigation of Legionnaires' Disease Incidents. Euro Surveill. 2015;20(14):21087.https://www.ncbi.nlm.nih.gov/entrez/query.

fcgi?cmd=Retrieve&db=PubMed&list_uids=25884146&dopt=AbstractPMID: 25884146

8. Swaminathan B, Gerner-Smidt P, Ng L-K, Lukinmaa S, Kam K-M, Rolando S, et al. Building PulseNet International: an interconnected system of laboratory networks to facilitate timely public health recognition and response to foodborne disease outbreaks and emerging foodborne diseases. Foodborne Pathog Dis. 2006;3(1):36-50. DOI: 10.1089/fpd.2006.3.36 PMID: 16602978
9. Nadon C, Van Walle I, Gerner-Smidt P, Campos J, Chinen I, Concepcion-Acevedo J, et al. PulseNet International: Vision for the implementation of whole genome sequencing (WGS) for global food-borne disease surveillance. Euro Surveill. 2017;22(23):30544. DOI: 10.2807/1560-7917.ES.2017.22.23.30544
10. Oberle M, Wohlwend N, Jonas D, Maurer FP, Jost G, Tschudin-Sutter S, et al. The Technical and Biological Reproducibility of Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) Based Typing: Employment of Bioinformatics in a Multicenter Study. PLoS One. 2016;11(10):e0164260. DOI: 10.1371/journal.pone.0164260 PMID: 27798637
11. Schlaberg R, Chiu CY, Miller S, Procop GW, Weinstock G, Professional Practice Committee and Committee on Laboratory Practices of the American Society for Microbiology, Microbiology Resource Committee of the College of American Pathologists. Validation of Metagenomic Next-Generation Sequencing Tests for Universal Pathogen Detection.Arch Pathol Lab Med. 2017;141(6):776-86. DOI: 10.5858/arpa.2016-0539-RA PMID: 28169558

License and copyright

Measles outbreak after 12 years without endemic transmission, Portugal, February to May 2017

F George [1], J Valente [1], GF Augusto [1], AJ Silva [1], N Pereira [1], T Fernandes [1], P Palminha [2], BA Aguiar [1], A Martins [1], E Santos [1], P Valente [1], E Calé [1], A Leça [1], PJ Nogueira [1]

1. Directorate-General of Health, Lisbon, Portugal
2. National Institute of Health Dr Ricardo Jorge, Lisbon, Portugal

Correspondence: Paulo Jorge Nogueira (paulo.nogueira@dgs.min-saude.pt)

Citation style for this article:
George F, Valente J, Augusto GF, Silva AJ, Pereira N, Fernandes T, Palminha P, Aguiar BA, Martins A, Santos E, Valente P, Calé E, Leça A, Nogueira PJ. Measles outbreak after 12 years without endemic transmission, Portugal, February to May 2017. Euro Surveill. 2017;22(23):pii=30548. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2017.22.23.30548

Article submitted on 30 May 2017 / accepted on 08 June 2017 / published on 08 June 2017

We report a measles outbreak in two Portuguese health regions (Algarve and Lisbon and the Tagus Valley) since February 2017, and which by 31 May resulted in 28 confirmed cases, of which 16 were unvaccinated. Thirteen cases were healthcare workers. One unvaccinated teenager died. Genotype B3 was identified in 14 cases from both regions. This outbreak occurs after 12 years without endemic measles transmission, and in a context of high measles vaccination coverage and immunity.

We describe a measles outbreak that started in February 2017, with 28 confirmed cases, as at 31 May. The investigation is ongoing and we present here preliminary findings and the implemented control measures. After the identification of the first measles case, a contingency plan at the Directorate-General of Health (Direção-Geral da Saúde, DGS) was implemented, following the Portuguese National Programme for Measles Elimination (Programa Nacional de Eliminação do Sarampo, PNES) [1]. This report is based on data extracted on 31 May from the National System for Epidemiological Surveillance (Sistema Nacional de Vigilância Epidemiológica, SINAVE), which is an integrated clinical and laboratory system of mandatory notification.

Vaccination against measles is included in the national immunisation programme (Programa Nacional de Vacinação, PNV) since 1974 and is available for free. This outbreak occurs after a 12-year period without endemic measles transmission in Portugal, which led the World Health Organization (WHO) Regional Office for Europe to certify measles as eliminated in the country in 2015 and 2016 [2]. In Portugal, measles is a mandatory notifiable disease since 1987 and, within the scope of the PNES [1], any suspected measles case notified is investigated thoroughly.

Immunity against measles is high in Portugal. The National Serological Survey conducted in 2001–02 showed a proportion of immune individuals above 93.4% in all age groups [3]. Preliminary results from the latest survey (2015–16), show that this trend of high immunity was maintained (data not shown).

Case definition

The measles case definition used during this outbreak was based on the European Commission case definition [4]. Measles cases were defined as possible, probable or confirmed, depending on clinical, epidemiological and laboratory criteria.

A possible case was any person who met clinical criteria i.e. fever, maculopapular rash, and any of cough/coryza/conjunctivitis; a probable case was any person who met clinical criteria and had an epidemiological link to a confirmed case; a confirmed case was any possible case with laboratory evidence of infection with measles virus i.e. detection of viral RNA in a biological sample and/or a positive IgM result in serum, determined by the WHO-certified national reference laboratory for measles and rubella (National Institute of Health – Instituto Nacional de Saúde Doutor Ricardo Jorge, INSA) [5].

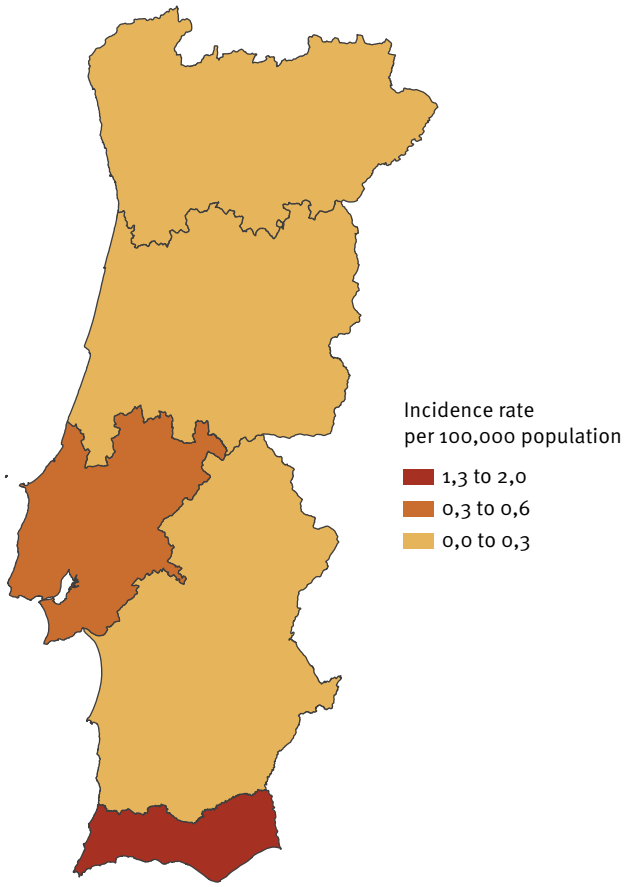
Following the WHO criteria [6], cases were discarded when clinical, epidemiological or laboratory criteria were not met, taking into account vaccination history and risk of measles infection in the community or abroad.

Outbreak description

As at 31 May, 156 measles cases were notified in Portugal in 2017. In the outbreak reported here, 28 cases were confirmed, seven were classified as possible, three cases were under investigation and 117 cases were discarded. Additionally, one confirmed imported measles case was identified in the north health region,

Figure 1

Incidence rate of confirmed measles cases per 100,000 population by health region, Portugal, 23 February–31 May 2017 (n = 28 confirmed cases)



Map developed by GeoSaúde (http://www.geosaude.dgs.pt/).

Source: Direção-Geral da Saúde, Sistema Nacional de Vigilância Epidemiológica.

which was an isolated case with no epidemiological or genotypic links to the cases in the outbreak described here.

All possible measles cases notified were investigated and control measures were promptly implemented in order to contain transmission. This report focuses on the description of confirmed cases in Algarve and Lisbon and the Tagus Valley (Lisboa e Vale do Tejo, LVT), two health regions which are not in close proximity (Figure 1).

The distribution of cases over time is described in the epidemic curve (Figure 2).

Algarve health region

The first measles case was notified to health authorities on 30 March (rash onset on 21 March), and was from the Algarve health region, a touristic region with tourists mainly from other European countries. Local and regional public health teams immediately initiated epidemiological investigations that led to the retrospective identification of three additional cases, with prior disease onset. Overall, this transmission chain comprised seven confirmed cases (Figure 1, 2). The earliest case had rash onset on 23 February and the latest had rash onset on 8 April. No further confirmed cases were identified in Algarve. Five of these cases acquired measles in a healthcare setting, including two healthcare workers; five cases (4 infants aged under 1 year and 1 adult) were hospitalised. To date, no epidemiological link has been found between this cluster and other cases reported in Portugal or abroad.

Lisbon and the Tagus Valley health region

The first case identified in the LVT health region was reported by a paediatrician from Cascais Hospital to the Regional Department of Public Health and the DGS on 6 April (rash onset on 30 March). Twenty-one cases were confirmed in LVT health region, with the earliest case having had rash onset on 17 March. This case was identified due to the epidemiological investigation of the first notified case in this region. The last confirmed case in the LVT region had developed rash on 13 May and no further measles cases were confirmed. The transmission chain in the LVT region is under investigation, with epidemiological links already identified between most cases. Eleven cases were healthcare workers. In the LVT region, seven cases were hospitalised, and one death has occurred in an unvaccinated teenager.

Characteristics of cases

Most confirmed measles cases (n = 19) occurred in adults (≥ 18 years), two cases were adolescents (10–18 years), and seven cases occurred in children (< 10 years) (Figure 3).

Of note, 13 cases occurred in healthcare workers (Table). Of the 28 cases, 16 had not been previously vaccinated; while the remaining cases had documented evidence of one (4 cases) or two or more doses (8 cases, including 1 case vaccinated with 3 doses, the first one between 6 and 12 months of age) of a measles vaccine, either single or combined (Table).

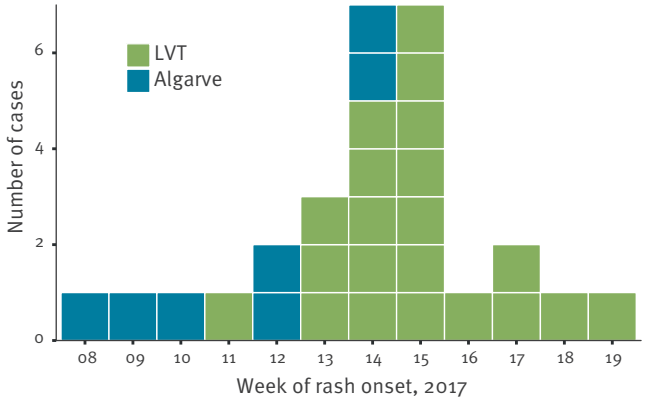
Among the unvaccinated cases, five were infants under 1 year of age and thus too young to be vaccinated, one case was 13 months old, two were adolescents, and the remaining eight cases were in adults (Figure 3). Thirteen cases were healthcare workers, of which three were unvaccinated.

Laboratory investigation

Laboratory investigation was carried out by INSA. A variety of samples were used to confirm or discard a measles case: serum, oral fluids or throat swabs, or urine. Measles cases were confirmed by positive IgM, detection of measles nucleic acid or isolation of measles virus.

Figure 2

Confirmed measles cases by week of rash onset, Portugal, 23 February–31 May 2017 (n = 28)

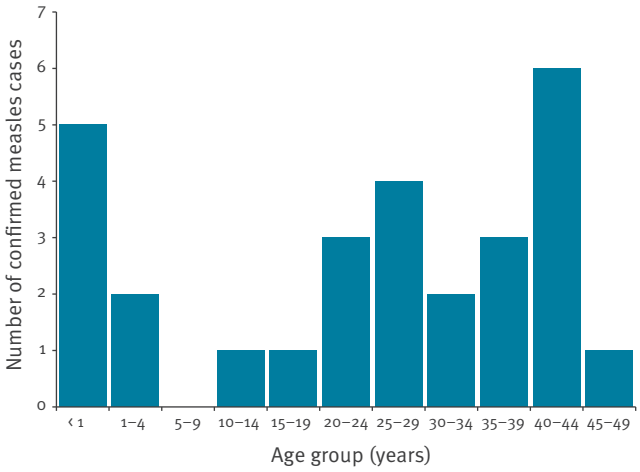


LVT: Lisboa e Vale do Tejo (Lisbon and the Tagus Valley).

Source: Direção-Geral da Saúde, Sistema Nacional de Vigilância Epidemiológica.

Figure 3

Confirmed measles cases by age group, Portugal, 23 February–31 May 2017 (n = 28)



Genotype B3 was identified in 14 cases from Algarve and LVT health regions, identical to the genotype detected in other outbreaks in Europe in 2017 e.g. in Belgium, Italy and the United Kingdom [7,8]. Genotyping of the remaining 14 confirmed cases is still ongoing.

Control measures

Following the increasing number of measles cases reported in several European countries in early 2017, DGS issued a warning to healthcare services, followed by recommendations and guidelines about diagnosis, early detection and response to measles cases, within the scope of the PNES [1].

In Portugal, when a suspected measles case is identified by health services, the physician is requested to fill in an electronic notification form at the SINAVE, and an email alert is simultaneously generated for local, regional and national public health authorities. Likewise, INSA also notifies DGS of all requests for measles tests, and notifies results electronically through SINAVE. Additionally, local public health units must inform immediately by phone and/or email regional and national public health authorities.

In this outbreak, all reported suspected measles cases were investigated and control measures were promptly implemented in order to contain transmission. While transmission could be prevented for imported case identified in the north health region, cases in Algarve and LVT had already generated transmission chains by the time they were reported.

Local public health teams undertook extensive contact tracing for all measles cases. Furthermore, surveillance and control measures included: immediate isolation of suspected cases, verification of immunisation status of close contacts, and administration of prophylactic immunoglobulin or measles-mumps-rubella (MMR) vaccine.

Epidemiological investigations and isolation of cases was complemented with broader public health measures, that included: (i) dissemination of key documents – posters about measles clinical picture, guidelines, epidemiological bulletins, and background materials for healthcare services – to support prevention and control measures [9-12]; (ii) creation of a specific section on measles on the DGS website [13], together with emails to inform healthcare professionals and schools; (iii) raising public awareness about the importance of vaccination through numerous reports in the media; (iv) enhancing vaccination, especially in children < 18 years and in healthcare workers, according to the PNV and PNES guidelines.

Discussion

As at 31 May, 28 laboratory-confirmed measles cases were identified in Portugal in 2017. Transmission occurred in three different settings: household, community and healthcare services. Besides interviewing patients, hospital staff and family members, public health authorities had to liaise with airline companies and foreign public health authorities, as one confirmed case travelled abroad during the incubation period.

This measles outbreak in a country with high levels of reported vaccination coverage and immunity [3,14] represents a challenge for the Portuguese public health authorities. In contrast with many European countries, Portugal records high uptake of MMR vaccine. However, immunity gaps persist, with vulnerable population pockets [3,14,15]. We found a relatively large proportion of measles cases in vaccinated individuals and this has also been reported in other outbreaks [16-18]. This situation is expected in highly vaccinated communities and might be explained by the fact that MMR is not 100% effective, with around 7.5% and

Table

Characteristics of confirmed measles cases, Portugal, 23 February–31 May 2017 (n = 28)

Characteristic	Total (n = 28)
Sex	
Male	12
Female	16
Occupation	
Physicians	5
Nurses	5
Other healthcare workers	3
Other occupation / NA	15
Vaccination status	
2 or more doses	8
1 dose	4
Not vaccinated	16

NA: not applicable.

5.0% non-respondents to the first and second doses, respectively [19].

Mass immunisation campaigns against measles took place in Portugal between 1973 and 1977. In 1974, the single measles vaccine was introduced in the PNV for children aged 12–15 months, and in 1987 the combined MMR. Since 1990, two doses of MMR vaccine are recommended for children. Since 2017, the recommended schedule is at 12 months and 5 years of age [20]. Two doses are also recommended for healthcare professionals, and one dose for adults born in or after 1970 without previous measles history [20]. Portugal has achieved sustained immunisation coverage against measles, above 95% for one and two doses in < 18-year-olds [21]. Measles immunity in older age groups was above 93% in 2002 [3].

Some delay between disease onset and notification in the early measles cases in Algarve and LVT could be explained by the fact that physicians might not have considered measles as the first diagnosis. This is very likely in a country without endemic measles transmission for more than a decade. After the first notifications, public health teams retrospectively investigated all potential measles cases, leading to the identification of four additional cases that had not been notified timely. The outbreak however made physicians more aware of the measles diagnosis and subsequent cases were notified timely.

The accumulation of measles-susceptible population pockets in a context of increasing number of outbreaks in European countries since 2016 [22,23] could have contributed to this outbreak in our country. Thus, possible links between these cases and other outbreaks in Europe are under investigation. The outbreak has increased national awareness and knowledge about measles diagnosis and epidemiological investigations, enhanced vaccination activities at the local level, and also motivated demand for vaccination from the general population and healthcare workers. The high and increasing number of notified possible measles cases being discarded in the course of this outbreak, increases confidence about its control. The last confirmed measles case in this outbreak was notified in the LVT health region on 14 May (rash onset on 13 May). No further confirmed cases linked to this case have been notified to date.

Acknowledgements

The authors wish to thank all members of local public health units, regional public health departments who are involved in investigation and control measures in this outbreak. Special thanks go also to all doctors, nurses and other healthcare professionals in hospitals and primary care units who have assisted with control measures, as well as all patients and their families who promptly cooperated in the epidemiological investigations.

Conflict of interest

None declared.

Authors' contributions

FG directed and AJS and PJN coordinated the investigations of the outbreak. GFA, JV, BAA, AM, NP, TF, PV and AL contributed to data collection, case information and data analysis. JV and GFA drafted the manuscript, with contribution by NP, TF, PV and AL. NP, TF, PP, ES, PV, AL and EC were involved in revising the manuscript. All authors reviewed and approved the final version.

References

1. Republic of Portugal – Directorate-General of Health (DGS). Programa Nacional para a Eliminação do Sarampo [National Programme for Measles Elimination]. Lisbon: DGS; Mar 2013. Portuguese. Available from: https://www.dgs.pt/documentos-e-publicacoes/programa-nacional-de-eliminacao-do-sarampo-jpg.aspx
2. World Health Organization (WHO) Regional Office for Europe. 4th Meeting of the European Regional Verification Commission for Measles and Rubella Elimination (RVC). Copenhagen: WHO; 2016. Available from: http://www.euro.who.int/__data/assets/pdf_file/0011/304958/4th-RVC-meeting-report.pdf?ua=1
3. Republic of Portugal – Directorate-General of Health (DGS). Avaliação do programa nacional de vacinação e melhoria do seu custo-efetividade: 2.º inquérito serológico nacional: Portugal Continental 2001-2002. [Evaluation of the National Immunisation Programme and improvement of its cost-effectiveness: 2nd National Serological Survey – Continental Portugal 2001-2002]. Lisbon: DGS; 2004. Portuguese. Available from: www.dgs.pt/ficheiros-de-upload-1/2-inquerito-serologico-nacional-livro-pdf.aspx
4. European Commission. Commission Implementing Decision of 8 August 2012 amending Decision 2002/253/EC laying down case definitions for reporting communicable diseases to the Community network under Decision No 2119/98/EC of the European Parliament and of the Council. Luxembourg: Publications Office of the European Union. L262 Sep 27, 2012. Available from: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ%3AL%3A2012%3A262%3ATOC
5. Republic of Portugal. Directorate-General of Health (DGS). Despacho N.º 15385-A/2016 [Dispatch No. 15385-A/2016]. In Diário da República, Serie II; 21 Dec 2016. Portuguese. Available from: https://dre.pt/home/-/dre/105574339/details/maximized?serie=II&dreId=105574337

6. World Health Organization (WHO). Eliminating Measles and rubella - Framework for the verification process in the WHO European Region 2014. Copenhagen: WHO; 2014. Available from: http://www.euro.who.int/__data/assets/pdf_file/0009/247356/Eliminating-measles-and-rubella-Framework-for-the-verification-process-in-the-WHO-European-Region.pdf
7. Grammens T, Schirvel C, Leenen S, Shodu N, Hutse V, Mendes da Costa E, et al. Ongoing measles outbreak in Wallonia, Belgium, December 2016 to March 2017: characteristics and challenges. Euro Surveill. 2017;22(17):30524. DOI: 10.2807/1560-7917.ES.2017.22.17.30524 PMID: 28488998
8. World Health Organization (WHO). Measles Nucleotide Surveillance (MeaNS) Database. Geneva: WHO. [Accessed 5 Jun 2017]. Available from: http://www.hpa-bioinformatics.org.uk/Measles/Public/Web_Front/main.php
9. Republic of Portugal – Ministry of Education and Ministry of Health. Despacho N.o 3668-A/2017 [Dispatch No. 3668-A/2017]. In Diário da República, II Serie 28 Apr 2017. Portuguese. Available from: https://dre.pt/application/file/a/106955144
10. Republic of Portugal – Directorate-General of Health (DGS). Sarampo: Procedimentos em Unidades de Saúde. [Measles: Procedures in Healthcare Units]. Lisbon: DGS. Apr 2017; Normative Document No. 4/2017. Portuguese. Available from: http://www.dgs.pt/saude-publica1/sarampo.aspx
11. Republic of Portugal – Directorate-General of Health (DGS). Sarampo. Informações à Comunidade Educativa [Measles. Informations to Schools]. Lisbon: DGS; Apr 2017. Guideline No. 6/2017. Portuguese. Available from: https://www.dgs.pt/directrizes-da-dgs/orientacoes-e-circulares-informativas/orientacao-n0062017-de-19042017-.aspx
12. Republic of Portugal – Directorate-General of Health (DGS). Sarampo. Medidas Especiais [Measles. Special Measures]. Lisbon: DGS; Apr 2017. Guideline No. 7/2017. Portuguese. Available from: www.dgs.pt/directrizes-da-dgs/orientacoes-e-circulares-informativas/orientacao-n0072017-de-20042017-pdf.aspx
13. Republic of Portugal – Directorate-General of Health (DGS). Sarampo. [Measles]. Lisbon: DGS. [Accessed 5 Jun 2017]. Portuguese. Available from: http://www.dgs.pt/saude-publica1/sarampo.aspx
14. World Health Organization (WHO). WHO vaccine-preventable diseases: monitoring system. Geneva: WHO. [Accessed 5 Jun 2017]. Available from: http://apps.who.int/immunization_monitoring/globalsummary
15. Republic of Portugal – Administration of the Lisbon and the Tagus Valley Health Region (ARSLVT). Perfil de Saúde. [Health Profile]. Lisbon: ARSLVT; 2015. Portuguese. Available from: http://www1.arslvt.min-saude.pt/Documents/ARS_Perfil%20de%20Saude_Final.pdf
16. Hahné SJ, Nic Lochlainn LM, van Burgel ND, Kerkhof J, Sane J, Yap KB, et al. Measles Outbreak Among Previously Immunized Healthcare Workers, the Netherlands, 2014. J Infect Dis. 2016;214(12):1980-6. DOI: 10.1093/infdis/jiw480 PMID: 27923955
17. Muscat M, Marinova L, Mankertz A, Gatcheva N, Mihneva Z, Santibanez S, et al. The measles outbreak in Bulgaria, 2009-2011: An epidemiological assessment and lessons learnt. Euro Surveill. 2016;21(9):30152. DOI: 10.2807/1560-7917.ES.2016.21.9.30152 PMID: 26967661
18. Moghadam M, Afsarkazerooni P, Ebrahimi M, Soltani M, Razmpoor A, Pirasteh E, et al. Measles outbreak in South of Iran, where vaccine coverage was high: a case-series study. Iran J Public Health. 2014;43(3):375-80.PMID: 25988100
19. World Health Organization. Measles vaccines: WHO position paper – April 2017.Wkly Epidemiol Rec. 2017;92(17):205-27. PMID: 28459148
20. Republic of Portugal – Directorate-General of Health (DGS). Programa Nacional de Vacinação 2017. [National Immunisation Programme 2017]. Lisbon: DGS; 2016. Portuguese. Available from: https://www.dgs.pt/documentos-e-publicacoes/programa-nacional-de-vacinacao-2017-pdf.aspx
21. Republic of Portugal. Directorate-General of Health (DGS), Directorate of Disease Prevention and Health Promotion. Programa Nacional de Vacinação. Boletins de Vacinação N.º 1, 6-8, 10. [National immunisation programme. Vaccination Bulletins No. 1, 6-8, 10]. Lisbon: DGS; [Accessed 7 Jun 2017]. Portuguese. Available from: https://www.dgs.pt/paginas-de-sistema/saude-de-a-a-z/programa-nacional-de-vacinacao/relatorios-e-publicacoes.aspx
22. European Centre for Disease Prevention and Control (ECDC). Communicable Disease Threats Report. Week 19, 7-13 May 2017. Stockholm: ECDC; May 2017. Available from: http://ecdc.europa.eu/en/publications/Publications/Communicable-disease-threats-report-13-may-2017.pdf
23. European Centre for Disease Prevention and Control (ECDC). Measles and rubella monitoring, January 2017 - Disease surveillance data: 1 January 2016 – 31 December 2016. Stockholm: ECDC; 2017. Available from: http://ecdc.europa.eu/en/publications/Publications/measles%20-rubella-monitoring-170424.pdf

License and copyright

Necrotising fasciitis as atypical presentation of infection with emerging *Neisseria meningitidis* serogroup W (MenW) clonal complex 11, the Netherlands, March 2017

A Russcher [1], E Fanoy [2 3], GDJ van Olden [4], AD Graafland [5], A van der Ende [6], MJ Knol [3]

1. Department of Medical Microbiology, Meander Medical Centre, Amersfoort, the Netherlands
2. Public Health Service Region Utrecht, Zeist, the Netherlands
3. Centre for Infectious Diseases Control, National Institute for Public Health and the Environment, Bilthoven, the Netherlands
4. Department of Surgery, Meander Medical Centre, Amersfoort, the Netherlands
5. Department of Intensive Care, Meander Medical Centre, Amersfoort, the Netherlands
6. Department of Medical Microbiology and the Netherlands Reference Laboratory for Bacterial Meningitis, Academic Medical Center, Amsterdam, the Netherlands

Correspondence: Anne Russcher (a.russcher@meandermc.nl)

Citation style for this article:
Russcher A, Fanoy E, van Olden GDJ, Graafland AD, van der Ende A, Knol MJ. Necrotising fasciitis as atypical presentation of infection with emerging Neisseria meningitidis serogroup W (MenW) clonal complex 11, the Netherlands, March 2017. Euro Surveill. 2017;22(23):pii=30549. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2017.22.23.30549

Article submitted on 14 May 2017 / accepted on 30 May 2017 / published on 08 June 2017

In March 2017, a patient with necrotising fasciitis caused by *Neisseria meningitidis* serogroup W (MenW) clonal complex 11 was diagnosed in the Netherlands. Unusual and severe presentations of MenW infections are common in the current European epidemic. In the Netherlands, the incidence of MenW infections increased 10-fold, from an average of 0.03 per 100,000 population in 2002–2014 to 0.29 in 2016. Awareness of atypical presentations enables timely adequate treatment and public health action.

Case description and microbiological findings

In March 2017 (day 0), a man in his early 60ies consulted his general practitioner (GP) because of a painful, red and swollen ankle since 1 day. Five days before his GP visit, he had experienced a fever that lasted 2 days and was accompanied by nausea and vomiting, from which he recovered spontaneously. The GP diagnosed a first episode of gout and prescribed a non-steroidal anti-inflammatory drug (NSAID). One day later (day 1), the patient visited the emergency department of a local hospital as the redness had spread and now covered his left lower leg up to the knee (Figure). Blistering was present on the ankle. Physical examination also revealed a red, painful area on his right elbow that the patient had been unaware of up until that moment. Prior to this illness, the patient had not travelled abroad, had generally been in good health and had an unremarkable medical history.

Emergency surgery was performed immediately because of the clinical suspicion of a necrotising fasciitis. Antibiotic treatment consisting of benzylpenicillin, 20 million units per day (MU/day) intravenously (IV), and clindamycin, 600 mg IV four times daily, was initiated. During surgery, extensive necrosis of subcutis and fascia of the lower leg was present and a fasciectomy of the total lower leg was necessary. Intraoperative Gram-staining of fascia tissue of both the leg and elbow showed the presence of Gram-negative diplococci and ceftriaxone, 2 g IV twice daily, was started.

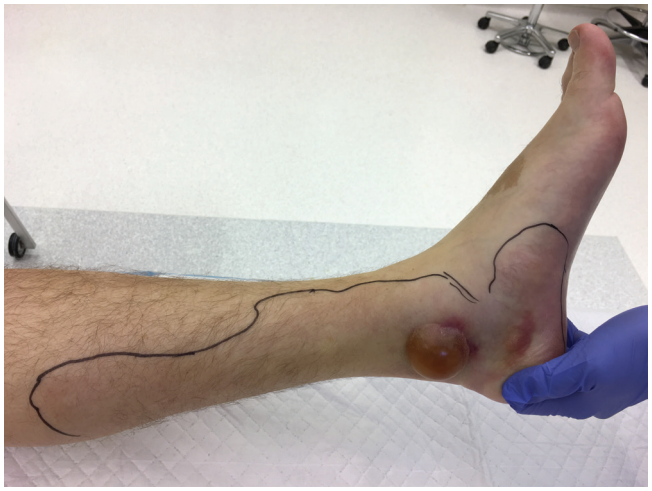
Post-operatively, the patient was admitted to the intensive care unit with septic shock and received circulatory support with noradrenalin and mechanical ventilation for 48 hours. A second-look operation within 12 hours after the first operation ensured that the first debridement had been sufficient. The following day, tissue cultures were positive for *Neisseria meningitidis*. Identification was performed by Maldi-ToF mass spectrometry (Bruker, Bremen, Germany). Susceptibility testing by E-test (Biomerieux, Marcy l'Etoile, France) with minimal inhibitory concentrations (MIC) in brackets showed susceptibility to penicillin (0.06 mg/L), ceftriaxone (0.04 mg/L), rifampicin (0.032 mg/L) and ciprofloxacin (0.008 mg/L) [1]. Typing at the Netherlands Reference Laboratory for Bacterial Meningitis revealed a serogroup W subtype P1.5,2:F1–1 belonging to the hypervirulent clonal complex 11.

Treatment and follow-up measures

When results of susceptibility testing became available, the patient was treated with benzylpenicillin, 20 MU/day, later lowered to 12 MU/day for another 7 days.

Figure 1

Blister on left ankle as clinical sign in case of necrotising fasciitis due to emerging *Neisseria meningitidis* serogroup W, the Netherlands, March 2017



Split skin grafting was performed on his leg. At the time of writing, the patient was recovering well.

The regional public health service (PHS) was contacted the day after admission (day 2) to evaluate the need for prophylactic antibiotic treatment and MenACWY vaccination of close contacts, but such measures were not needed as the patient had lived alone during the week before the onset of disease and there were no close contacts otherwise. The patient received a MenACWY vaccination to prevent a (re)infection in the following 3–5 years [2,3]. The case was notified by the PHS to the National Institute for Public Health and the Environment (RIVM) by the mandatory surveillance system. The Netherlands Early Warning Committee (NEWC) discussed the remarkable clinical presentation of this MenW case and communicated it to Dutch medical professionals via the weekly NEWC report.

Epidemiological situation in the Netherlands

Before 2015, the incidence of MenW disease was very low in the Netherlands, with an average annual incidence of 0.03 per 100,000 population from 2002 to 2014 (range: 0.01–0.04 per 100,000 population). The incidence increased to 0.05 per 100,000 population (n = 9 cases) in 2015 and 0.29 per 100,000 population (n = 50 cases) in 2016 [4]. The increase started in October 2015 and up until 1 April 2017, 79 MenW cases were reported. In 74 of 79 cases, the finetype could be determined (1 PCR-positive, 73 cultured isolates). It was P1.2,5:F1–1 in 68 of 74 cases (92%). This finetype is associated with hypervirulent clonal complex 11 [5]. Of the remaining five cases (all PCR-positive, culture-negative), not enough material was available to perform typing. MenW incidence was highest among persons aged 65 years or older (0.65/100,000; n = 30), followed by 15–24-year-olds (0.48/100,000; n = 15). Of 79 MenW cases, nine died (11%): four of them were 15–24 years old, two were 45–64 years old and three were 65 years or older. The clinical manifestation was known for 71 cases: 32 had septicaemia (45%), 14 had meningitis (20%), and seven had both septicaemia and meningitis (10%). The other cases had other clinical manifestations including bacteraemic pneumonia (n = 12; 17%), septic arthritis (n = 4; 6%), pericarditis (n = 1) and necrotising fasciitis (n = 1, the case described above). To our knowledge, three patients with septicaemia, one of whom died, presented predominantly with gastrointestinal symptoms. None of the cases were epidemiologically related and there is no geographical clustering.

Discussion

Infections due to MenW may present differently from the classical clinical presentation of *N. meningitidis* such as meningitis or septicaemia; for example, gastrointestinal presentations have been reported with MenW [6,7]. Necrotising fasciitis can be caused by various bacterial pathogens, but in monomicrobial infections, haemolytic *Streptococci* group A are most commonly identified as the cause [8]. *N. meningitidis* as the causative agent in necrotising fasciitis is extremely rare [9], although there are some reports on severe cellulitis being caused by *N. meningitidis* [10,11].

In the reported case, the presence of infectious foci on both the leg and arm strongly suggests haematogenous spread, probably from nasopharyngeal carriage as no other apparent focus was identified. Blood cultures remained negative, but these were mistakenly drawn after antimicrobial treatment had already been started. The preceding gastrointestinal symptoms could have been related to the MenW infection as was recently described by others [6,7], but they might be a remarkable coincidence as the necrotising fasciitis symptoms did not develop until 3 days later. Reported cases of MenW with gastrointestinal symptoms quickly progressed into septicaemia [7].

Other European countries have also reported MenW cases due to this hypervirulent strain [5,12].

A similar increase in incidence and a similar age distribution in MenW cases has been noted in the United Kingdom (UK) starting in the epidemiological year 2009/2010. Campbell et al. report a case fatality rate of 12% (21/170) in 2014/15 [12]. A similar case fatality rate of 12% (15/129) was reported for the previous period 2010/11-2012/13 (three epidemiological years) [13]. These findings led to emergency vaccination of 13 to 18-year-olds with a MenACWY vaccine [14]. The first results of this programme seem promising, with an observed 69% reduction of predicted cases in the cohort offered vaccination at a vaccination coverage of only 37% [15]. Although this particular case of necrotising fasciitis developed in a patient in their 60ies, introduction of a vaccination programme against MenW in the Netherlands and possibly other European countries

might be justified based on the comparable epidemiological pattern and high case fatality rate.

In conclusion, our case underscores the unusual presentation and severity of MenW infections, mostly caused by the hypervirulent clonal complex 11, in the current epidemic. Physicians should be vigilant for rare presentations of MenW infections considering the necessity of prompt diagnosis for optimal treatment. Based on the current epidemiology, inclusion of vaccination against MenW disease in the Dutch national immunisation programme should be discussed.

Acknowledgements

This work was not funded by external sources.

Conflict of interest

None declared.

Authors' contributions

AR, EF and MK wrote the first draft of the manuscript. All authors critically read and revised the manuscript. AR, GvO and AG participated in clinical management of the patient. AR and AvdE were responsible for laboratory tests. EF was responsible for public health policies. MK and AvdE collected and analysed all MenW cases in the Netherlands.

References

1. The European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoints tables for interpretation of MICs and zone diameters, Version 7.1, Växjö: EUCAST. 2017. Available from: http://www.eucast.org
2. Baxter R, Reisinger K, Block SL, Izu A, Odrljin T, Dull P. Antibody persistence and booster response of a quadrivalent meningococcal conjugate vaccine in adolescents.J Pediatr. 2014;164(6):1409-15. DOI: 10.1016/j.jpeds.2014.02.025 PMID: 24657122
3. Cohn AC, MacNeil JR, Harrison LH, Lynfield R, Reingold A, Schaffner W, et al. Effectiveness and Duration of Protection of One Dose of a Meningococcal Conjugate Vaccine. Pediatrics. 2017;139(2):e20162193. DOI: 10.1542/peds.2016-2193 PMID: 28100689
4. Knol M, Ruijs WLM, Melker HE, Berbers GAM, van der Ende A. Sudden increase of invasive meningococcal disease serogroup W in 2015 and 2016 [Plotselinge toename van invasieve meningokokkenziekte serogroep W in 2015 en 2016]. Infectieziekten Bulletin. 2017;28(1):23-8. Dutch. Available from: http://www.rivm.nl/dsresource?objectid=9fcfee07-c3dc-4af8-9437-7e328a66469c&type=pdf&disposition=inline
5. Lucidarme J, Scott KJ, Ure R, Smith A, Lindsay D, Stenmark B, et al. An international invasive meningococcal disease outbreak due to a novel and rapidly expanding serogroup W strain, Scotland and Sweden, July to August 2015. Euro Surveill. 2016;21(45):30395. DOI: 10.2807/1560-7917.ES.2016.21.45.30395 PMID: 27918265
6. Wunderink HF, Vlasveld I, Knol MJ, van der Ende A, van Essen E, Kuijper E. Diarree en sepsis door Neisseria meningitidis serogroep W.[Diarrhoea and septicaemia caused by Neisseria meningitidis serogroup W].Ned Tijdschr Geneeskd. Dutch. Forthcoming.
7. Campbell H, Parikh SR, Borrow R, Kaczmarski E, Ramsay ME, Ladhani SN. Presentation with gastrointestinal symptoms and high case fatality associated with group W meningococcal disease (MenW) in teenagers, England, July 2015 to January 2016.Euro Surveill. 2016;21(12):30175. DOI: 10.2807/1560-7917.ES.2016.21.12.30175 PMID: 27035055
8. Pasternack MS, Schwartz MN. Cellulitis, Necrotizing Fasciitis, and Subcutaneous Tissue Infections. In: Bennett JE, Dolin R, Blaser MJ, editors. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. 8th ed. Philadelphia: Elsevier Saunders, 2015: 1194-1215.
9. Mentec H, Chosidow O, Lafaurie P, Darmon JY, Simon M, Roujeau JC, et al. Fasciite nécrosante à Neisseria meningitidis atteignant simultanément le bras et la jambe.[Necrotizing fasciitis, caused by Neisseria meningitidis, simultaneously involving an arm and a leg]. Ann Dermatol Venereol. 1993;120(12):889-91. French.PMID: 8074348
10. Carrascosa MF, Casuso-Sáenz E, Salcines-Caviedes JR. Neisseria meningitidis cellulitis.Int J Infect Dis. 2012;16(10):e760. DOI: 10.1016/j.ijid.2012.04.014 PMID: 22727693
11. Ozaki B, Kittai A, Chang S. Neisseria meningitidis as a cause of facial cellulitis. BMJ Case Rep. 2014:bcr2014203774. DOI: doi:10.1136/bcr-2014-203774 .PMID: 24626385
12. Abad R, Vázquez JA. Early evidence of expanding W ST-11 CC meningococcal incidence in Spain.J Infect. 2016;73(3):296-7. DOI: 10.1016/j.jinf.2016.06.010 PMID: 27387450
13. Ladhani SN, Beebeejaun K, Lucidarme J, Campbell H, Gray S, Kaczmarski E, et al. Increase in endemic Neisseria meningitidis capsular group W sequence type 11 complex associated with severe invasive disease in England and Wales. Clin Infect Dis. 2015;60(4):578-85. DOI: 10.1093/cid/ciu881 PMID: 25389259
14. Campbell H, Saliba V, Borrow R, Ramsay M, Ladhani SN. Targeted vaccination of teenagers following continued rapid endemic expansion of a single meningococcal group W clone (sequence type 11 clonal complex), United Kingdom 2015. Euro Surveill. 2015;20(28):21188. DOI: 10.2807/1560-7917.ES2015.20.28.21188 PMID: 26212140
15. Campbell H, Edelstein M, Andrews N, Borrow R, Ramsay M, Ladhani S. Emergency meningococcal ACWY vaccination program for teenagers to control group W meningococcal disease, England, 2015-2016.Emerg Infect Dis. 2017;23(7). DOI: 10.3201/eid2307.170236 PMID: 28409739

PulseNet International: Vision for the implementation of whole genome sequencing (WGS) for global food-borne disease surveillance

Celine Nadon[1,2], Ivo Van Walle[2,3], Peter Gerner-Smidt[4], Josefina Campos[5], Isabel Chinen[5], Jeniffer Concepcion-Acevedo[4], Brent Gilpin[6], Anthony M. Smith[7], Kai Man Kam[8], Enrique Perez[9], Eija Trees[4], Kristy Kubota[10], Johanna Takkinen[3], Eva Møller Nielsen[11], Heather Carleton[4], FWD-NEXT Expert Panel[12]

1. Public Health Agency of Canada, National Microbiology Laboratory, Canada
2. These authors contributed equally to this work
3. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
4. Centers for Disease Control and Prevention, United States
5. National Institute of Infectious Diseases "Dr Carlos G. Malbran", Argentina
6. Institute of Environmental Science and Research Limited; Christchurch, New Zealand
7. National Institute for Communicable Diseases, South Africa
8. Chinese University of Hong Kong, Hong Kong Special Adminstrative Region, China
9. Pan American Health Organization/World Health Organization, Washington, DC, United States
10. Association of Public Health Laboratories, United States
11. Statens Serum Institut, Denmark
12. The members of the FWD-NEXT Expert Panel are listed at the end of the article

Correspondence: Celine Nadon (celine.nadon@phac-aspc.gc.ca)

Citation style for this article:
Nadon C, Van Walle I, Gerner-Smidt P, Campos J, Chinen I, Concepcion-Acevedo J, Gilpin B, Smith AM, Kam KM, Perez E, Trees E, Kubota K, Takkinen J, Nielsen EM, Carleton H, FWD-NEXT Expert Panel. PulseNet International: Vision for the implementation of whole genome sequencing (WGS) for global food-borne disease surveillance. Euro Surveill. 2017;22(23):pii=30544. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2017.22.23.30544

Article submitted on 26 January 2017 / accepted on 11 April 2017 / published on 08 June 2017

PulseNet International is a global network dedicated to laboratory-based surveillance for food-borne diseases. The network comprises the national and regional laboratory networks of Africa, Asia Pacific, Canada, Europe, Latin America and the Caribbean, the Middle East, and the United States. The PulseNet International vision is the standardised use of whole genome sequencing (WGS) to identify and subtype food-borne bacterial pathogens worldwide, replacing traditional methods to strengthen preparedness and response, reduce global social and economic disease burden, and save lives. To meet the needs of real-time surveillance, the PulseNet International network will standardise subtyping via WGS using whole genome multilocus sequence typing (wgMLST), which delivers sufficiently high resolution and epidemiological concordance, plus unambiguous nomenclature for the purposes of surveillance. Standardised protocols, validation studies, quality control programmes, database and nomenclature development, and training should support the implementation and decentralisation of WGS. Ideally, WGS data collected for surveillance purposes should be publicly available, in real time where possible, respecting data protection policies. WGS data are suitable for surveillance and outbreak purposes and for answering scientific questions pertaining to source attribution, antimicrobial resistance, transmission patterns, and virulence, which will further enable the protection and improvement of public health with respect to food-borne disease.

Introduction

Almost one in 10 people in the world become ill every year due to consumption of contaminated food; diarrhoeal diseases are the most common cause of illness, with 550 million cases and 230,000 deaths every year [1]. Children under five years of age bear 40% of this burden along with potentially life-long sequelae [1]. *Campylobacter jejuni/coli* and *Salmonella enterica* are the most common causes of bacterial diarrhoea globally and are responsible for ca 96 and 80 million infections every year, respectively [1].

PulseNet International is a global laboratory network dedicated to bacterial food-borne disease surveillance, comprised of the national, regional and subregional laboratory networks of Africa, Asia Pacific, Canada, Europe, Latin America and the Caribbean, the Middle East, and the United States (US); 86 countries in total (Figure 1) [2].

The mission of PulseNet International is to implement standardised genotyping methods and share information in real-time within regional and national laboratory networks to support surveillance and outbreak response enabling the direct comparison of inter-laboratory data irrespective of geography.

The primary method of molecular subtyping used by PulseNet International for the identification and investigation of outbreaks has been pulsed-field gel electrophoresis (PFGE), with multilocus variable-number

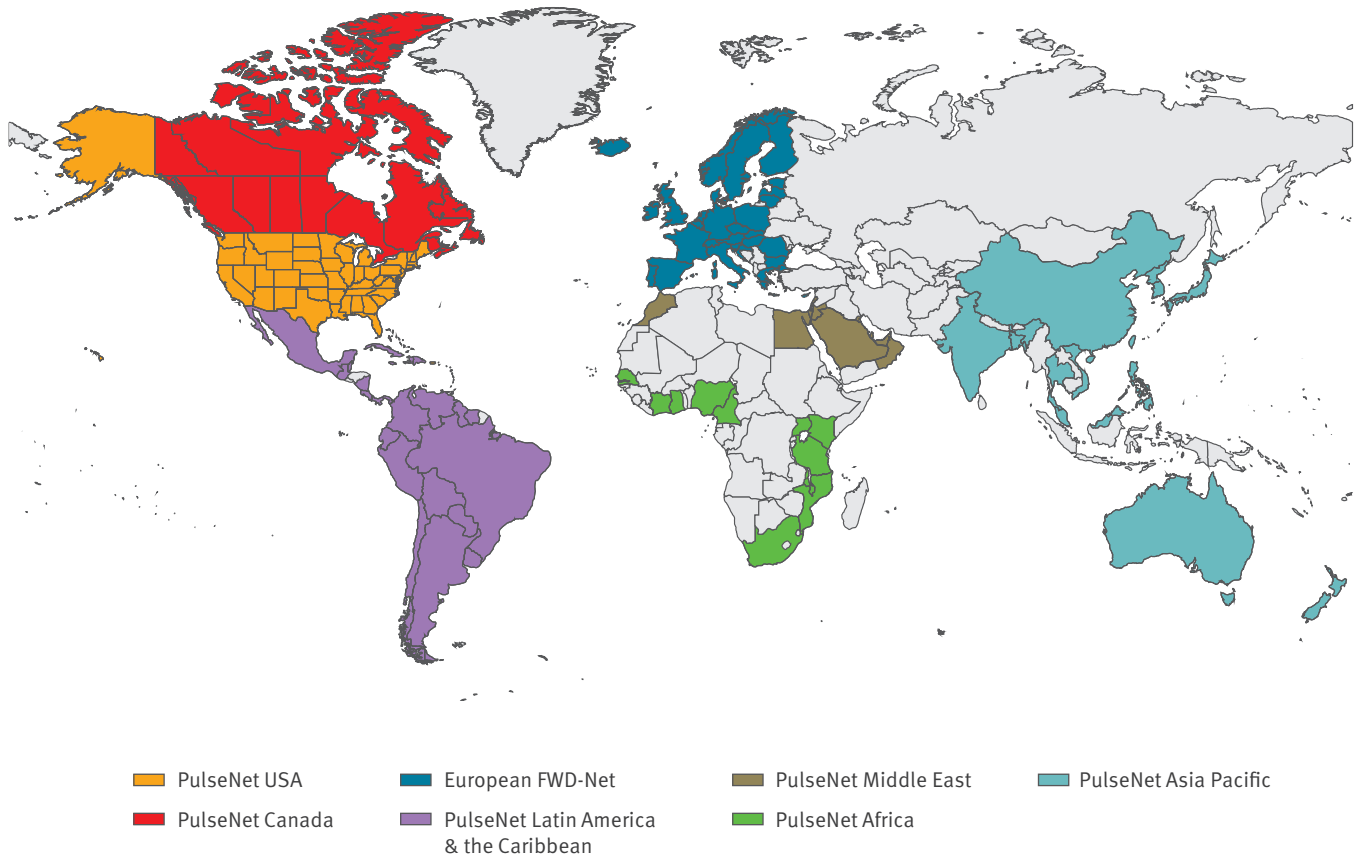
tandem repeat analysis (MLVA) applied to selected organisms [3]. The use of standardised, validated protocols and analysis procedures by all participants coupled with consistent interpretive criteria has enabled countless successful outbreak investigations, both within single countries and also spanning across borders [4]. Current PulseNet methods, PFGE and MLVA, are no longer considered cutting edge but have been extremely efficient in driving the detection, investigation and control of food-borne infection outbreaks in the past 20 years due to the demonstration of high typeability, reproducibility, discriminatory power, and good epidemiological concordance [5]. For timely and effective surveillance and outbreak response, data must remain comparable at all times among laboratories; any modifications on existing methods or introduction of new methods must be carefully validated and implemented by *all* network members in order to be effective and to avoid disrupting the surveillance due to backwards incompatibility issues [3,6]. Although new methods are often tested within the network they rarely make it into routine surveillance for this reason. Whole genome sequencing (WGS) has benefits that outweigh the challenges of disrupting the surveillance within the network.

WGS has shown superior sensitivity, specificity and more timely resolution to outbreak clustering compared with traditional methods [7-14]. Examples of the ability of WGS to facilitate emergency response are also demonstrated by International Food Safety Authorities Network (INFOSAN) emergency alerts; at least thirteen of the 48 biological events reported in 2014 and 2015 were supported with WGS [15]. Other strengths of WGS are its applicability to all organisms and its potential to provide multiple tests *in silico* from a single assay. These include subtyping tests, inferring biological properties (e.g. virulence genes, antibiotic resistance), and other phenotype predictions such as serotype. Moreover, genome sequencing elucidates the actual phylogenetic relationship among isolates. This renders the data useful for answering broader questions outside the relatively narrow scope of outbreak detection and response. For example, the same data used for routine surveillance could also be used for precise microbiological attribution studies, to elucidate transmission pathways and common properties of persistent strains, and to identify potential intervention points along the food safety continuum.

The vision of PulseNet International is for WGS to be used in all public health laboratories to identify, characterise and subtype food-borne pathogens, largely

Figure 1

Map of PulseNet International participating countries, May 2017



FWD: food- and waterborne disease; USA: United States.

replacing existing phenotypic and molecular methods in support of preparedness and response to food-borne illness at the local, national, regional and global levels. This paper provides considerations of the critical technical and practical aspects of WGS from the perspective of standardised international laboratory-based surveillance and the prerequisites for routine implementation in public health.

Whole genome sequence generation

Preparation and sequencing

There are several technologies available for genome sequencing. Collectively known as massively parallel sequencing, they produce billions of nt sequences during each run, where each genome is sequenced multiple times in small random pieces (reads and contigs) to generate very large datasets [16]. Even though sequencing platforms (e.g. Illumina, etc.) have different biochemistry and arrays, the workflow is similar: (i) DNA extraction; (ii) library preparation, which usually includes shearing the DNA either mechanically or enzymatically, adding adaptors and barcodes/indexes, and amplification; (iii) template preparation, either by bridge amplification or emulsion PCR; and (iv) sequencing (the sequencing run itself is highly automated).

Data processing

Processing data from WGS, regardless of the platform used, follows the same general workflow. Fragments or 'reads' (FASTQ format) from sequencing runs are trimmed to remove adaptor and barcode sequences (added during library generation), and low-quality reads. Depending on the analysis method chosen, the reads may or may not need to be assembled. Assembling is the process of placing all of the reads together in the correct order to create a small number of contiguous sequences, known as contigs (FASTA format). This can be done using a known reference sequence (closed genome) to guide the assembly, or it can be done de novo (draft genome), i.e. without prior knowledge of the expected order. Especially the latter is computationally relatively demanding for the four main species involved in food-borne diseases: *Salmonella* spp, *Campylobacter* spp, *Listeria monocytogenes* and Shiga toxin-producing *Escherichia coli* [17]. With current technologies, due to the short length of the individual reads compared with repetitive regions, a genome for these species is also rarely fully assembled (closed genome).

Analysis methods

K-mer, SNP, and gene-by-gene methods for *in silico* subtyping

There are many ways to analyse whole genome sequence data, including methods that will replace traditional molecular subtyping methods. Three common approaches for this are k-mers, single nucleotide polymorphisms (SNP), and the gene-by-gene based – i.e. extended multilocus sequence typing (MLST) based on WGS – approach. These methods have good epidemiological concordance, but differ in other features such as amenability to standardisation, stable nomenclature, scalability, the need for reference strains or assemblies, and computing and bioinformatics resource requirements (Table 1).

Briefly, k-mers have lower discriminatory capacity than the two other methods and are mainly useful for crude and rapid initial sequence comparison of isolates where maximum resolution is not needed and as a tool for detection of species [18]. The SNP approach is highly discriminatory but sensitive to the selection of the reference the SNPs are called against; this means that if two laboratories use a different reference for their SNP calling of the same outbreak the SNP differences of isolates not included in the analysis in both laboratories cannot be directly compared [18]. SNP analysis is computationally intensive and might therefore be rather slow; access to high performance computing is mandatory for analysing large sequence sets. Additionally, many SNP pipelines are currently command-line based, requiring substantial bioinformatics knowledge by the end-user. This is likely to change as the field progresses and pipelines continue to evolve to more user-friendly,

Table 1

Key features of k-mer, single nucleotide polymorphism (SNP) and multilocus sequence typing (MLST) approaches relevant to laboratories providing routine public health functions

Features	K-mer	SNP	MLST
Epidemiological concordance	Intermediate	High	High
Discrimination	Intermediate	High	High
Stable strain nomenclature	No	No	Yes
International standardisation	No	No	Yes
Scalability	No	No	Yes
Speed	Intermediate	Slow SNP calling, slow comparisons	Slow allele calling, fast comparisons
Local computing requirements	Low	High	Low
Local bioinformatics expertise required	Yes	Yes	No
Curation of database	No	No	Yes

SNP: single nucleotide polymorphisms; MLST: extended multilocus sequence typing.

'plug and play' formats. The method chosen for standardised surveillance by PulseNet International is the gene-by-gene approach.

Whole genome MLST, the method chosen by PulseNet International

For integration into routine public health surveillance and for maintaining inter-laboratory comparability, the gene-by-gene, i.e. extended MLST, approach offers a number of compelling features. The extended MLST schemes assess information from coding regions only, and collapse different types of mutations into a single allelic change. Allele information is assessed by comparing new sequences with an allele database that contains all genes ('loci') present in the typical several hundred strains used to create the scheme. The number of genes assessed may range from typically seven housekeeping genes to a several thousand [19]. The biggest schemes contain the genes in the core genome (genes present in nearly all strains of the same species, core genome (cg)MLST), or the whole or pan genome (all core genes plus accessory genes present in any strain used to create the allele database, whole genome (wg)MLST). Custom sets of any number of genes may also be analysed. Constructing and validating a reliable cg- or wgMLST scheme that is also accessible to all and simple to run is a significant undertaking but once implemented such schemes could be easy to work with by the end-users (microbiologists and epidemiologists). Nevertheless, the output from wgMLST analyses may also need consultation with experts to ensure proper interpretation. The allele calling process can be automated but is fairly slow; once alleles are called the analytical process is fast. In contrast to SNP analysis, wgMLST comparisons are much faster and may run on ordinary desktop computers, as an allelic profile is simply a string of integers.

Extended MLST schemes are phylogenetically relevant and at least in their most extensive form, wgMLST, they appear to be as discriminatory as SNP comparisons for *Salmonella* and *Listeria*, and provide significantly improved resolution and epidemiological concordance compared with molecular methods (data not shown).

Backwards compatibility of WGS with the existing gold standard methods PFGE and MLVA is very limited, regardless of the WGS analysis method. The short reads are not amenable to a complete assembly, which would be required to predict a PFGE restriction pattern, and in particular do not assemble the repetitive regions assessed by MLVA correctly. To mitigate the effects of losing comparability, some laboratories are choosing to perform both molecular and WGS-based subtyping in parallel for a period of time. The seven-gene MLST pattern can readily be extracted from WGS data.

The technical performance of wgMLST along with its scalability and amenability to standardisation and stable nomenclature (see section below) plus the computational and bioinformatics prerequisites realistic for many public health laboratories position wgMLST as the method of choice at this time for PulseNet International.

Implications for information technology (IT) infrastructure and bioinformatics expertise

WGS presents a number of challenges to the IT infrastructure of most PulseNet laboratories, which often operate in tightly regulated Windows-operating system-based computing environments and of which some may not always have stable power supply or Internet connections. Key issues for implementing WGS are bioinformatics expertise and software, and especially for larger laboratories storage space and computing power.

Storage space

Each sequencing run generates gigabytes (GB) of data, with sequence read sets (SRS) of 100–500 megabyte (MB) in size for each isolate. Many laboratories anticipate analysing thousands of isolates each year, and storage on existing systems will likely be prohibitively expensive. Generally speaking, it is desirable to retain the SRSs (and not just the final processed data assembled) so that they may be used for future analyses by using alternative methods such as SNPs or assessment of mobile genetic elements e.g. from phages. Some PulseNet International members currently store the SRSs within their own reference laboratory's data storage. Some also submit them in close to real-time to one of the databases of the International Nt Sequence Database Collaboration (INSDC, http://www.insdc.org), such as the Sequence Read Archive (SRA) at the National Center for Biotechnology Information and the European Nt Archive at the European Molecular Biology Laboratory, as well as the DNA Databank of Japan Sequence Read Archive (DRA). Using a public domain archive removes the storage cost from individual laboratories and makes the data available to the wider scientific community in perpetuity, but also necessitates public data sharing. This is problematic for some countries with respect to data protection (see section on data sharing below). Additionally, it is expected that very few countries in the future will have the capacity to store the ever growing amount of raw sequence data in-house. Strategies to manage the information associated with each isolate (i.e. the descriptive data, sometimes referred to as meta-data) are being developed and implemented by some PulseNet International network laboratories (see data sharing section below). Another potential storage solution consists of commercial clouds (e.g. BaseSpace, Amazon S3), with users paying per GB of storage; storage costs using these options are typically lower than in-house solutions; however, many organisations prohibit the use of commercial clouds due to institutional data security policies. Additionally, if an outside-house storage solution is pursued, Internet connections with adequate bandwidth must be available. Given the great diversity in resources across PulseNet International laboratories,

a one-size-fits-all solution might not be possible but renders publicly available storage options compelling.

Computing power, bioinformatics expertise, and software

Laboratories have three options for bioinformatics tools: in-house pipelines, web and/or cloud-based tools, and outsourcing; each has pros and cons. Many in-house bioinformatics pipelines utilise Linux-based tools, which while powerful, cannot be easily run on Windows- operating system-based computers and are frequently not supported by institutions' information technology (IT) departments.

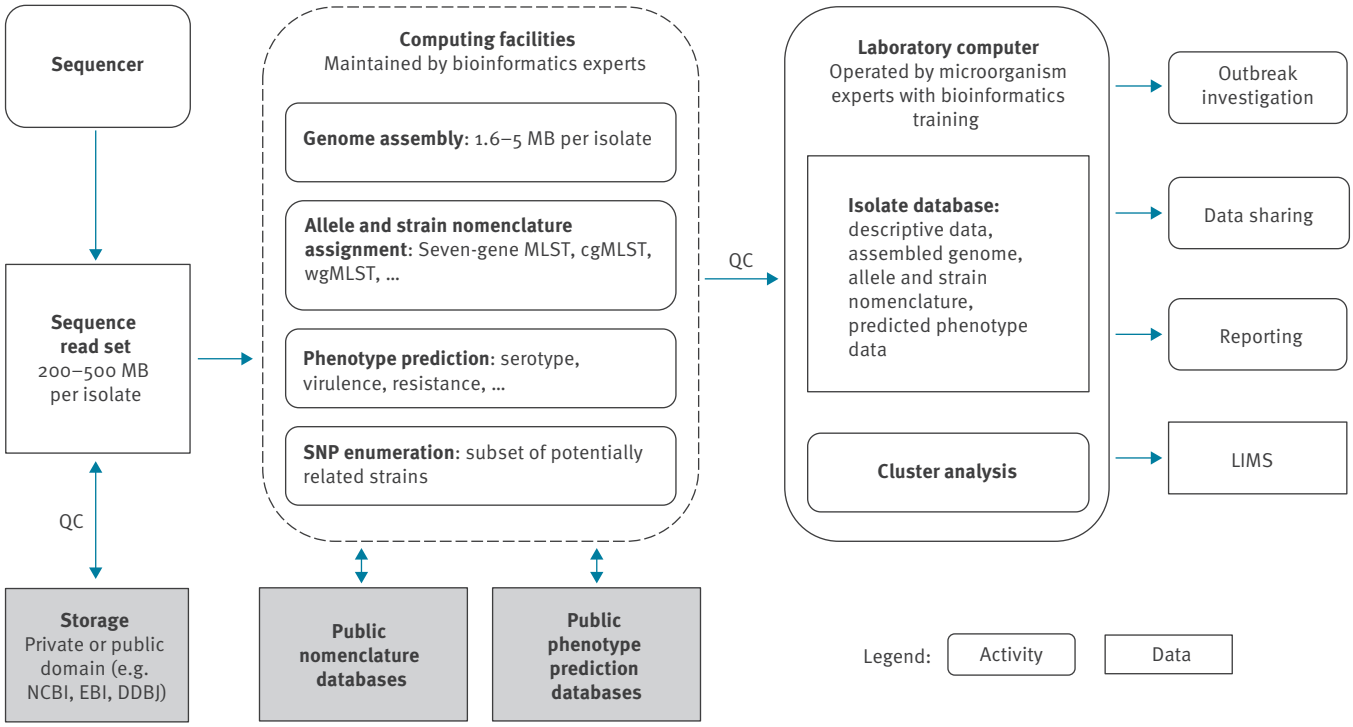
Cloud-based and publicly available computing and bioinformatics may provide a solution for analysis (e.g. the Center for Genomic Epidemiology, http://www.genomicepidemiology.org/). However, public health laboratories operate under strict quality assurance and accreditation requirements, necessitating guaranteed access to computing and bioinformatics tools. Within a single country or region, cloud-based bioinformatics tools can be maintained by local specialists, be partitioned from secure corporate or in-house computing networks as needed, and in many cases be scaled to meet demand (Figure 2). For PulseNet International laboratories with modest numbers of isolates, making use of cloud-based or other web-based public analysis tools may facilitate entry into WGS (as could outsourcing the actual sequencing instead of purchasing the equipment). The actual analysis tools can be used from open sources or commercial software.

Additionally, outsourcing analyses to a third party is an alternative, e.g. a PulseNet regional coordinator may serve as a third party analyst as it was done in PulseNet Latin America and the Caribbean for the analysis of PFGE data while waiting for individual participants to acquire the analytical software [2,20].

Due to the nature of public health and regulatory decision making action in food safety, robustness is essential as subtyping results often provide the critical evidence during food recalls, outbreak investigations, and prosecutions; they must be defendable in a court of law. For this reason, the ability to track the versions of software tools and parameters is a basic requirement, i.e. the ability to precisely recreate analyses from

Figure 2

Potential solutions for computing and storage in PulseNet International laboratories, May 2017



Cg: core genome; DDBJ: DNA Data Bank of Japan; EBI: European Bioinformatics Institute; LIMS: Laboratory Information Management System; MB: megabyte; MLST: multilocus sequence typing; NCBI: National Center for Biotechnology Information; QC: quality control; SNP: single nucleotide polymorphism; wg: whole genome.

This workflow separates storage of the sequence data, from the intensive bioinformatics analyses that need to be performed on them, in particular genome assembly and allele calling. This allows performing these analyses on a dedicated computing cluster either in-house or in the cloud, where they can be maintained and updated by bioinformatics experts who may not reside within the same organisation or even country as the PulseNet laboratory. No confidential information is handled on this computing cluster. Within the PulseNet laboratory, microorganism experts will perform quality controls, cluster analysis on MLST data, as well as evaluating virulence and other factors. Following data interpretation, laboratory experts will use this information for outbreak investigations, reporting and sharing with other agencies. When required, SNP analysis will be performed on subsets of closely related strains.

the past and arrive at the same results is a necessity. Presently, many PulseNet International laboratories do not have ready access to bioinformatics expertise. In deciding to use open source or commercial software, issues to consider include the need and ability to modify and tailor the source code, the ability of the non-bioinformatician PulseNet participants to learn and use the tools, the process for and cost of maintenance and updates, version control and other quality measures, and the cost of licenses and bioinformatics personnel. The ability to store and analyse associated descriptive and other laboratory data alongside the sequence data also needs to be considered.

Nomenclature

Despite achieving success in standardising molecular subtyping and inter-laboratory comparability across 86 countries, PulseNet International has not been able to implement global PFGE nomenclature for food-borne pathogens, largely due to the complexities (and costs) of implementing a central, global PFGE database requiring frequent manual curation. To date, each country or region maintains their own PFGE databases and nomenclature, with the exchange of data files to compare results across disparate nomenclatures as needed. In addition to its superior performance, WGS provides at long last the opportunity for a truly global nomenclature, facilitating laboratory-based surveillance at the international level and opening the door to future reductions in the burden of food-borne disease. Gene-by-gene-based approaches to WGS-based subtyping are highly suitable to stable nomenclature and, with international agreement to use the same scheme, provide a natural choice for worldwide implementation via PulseNet (see previous section). A detailed consideration of nomenclature is provided by the European food- and waterborne disease (FWD)-NEXT Expert Group [21].

Allele nomenclature

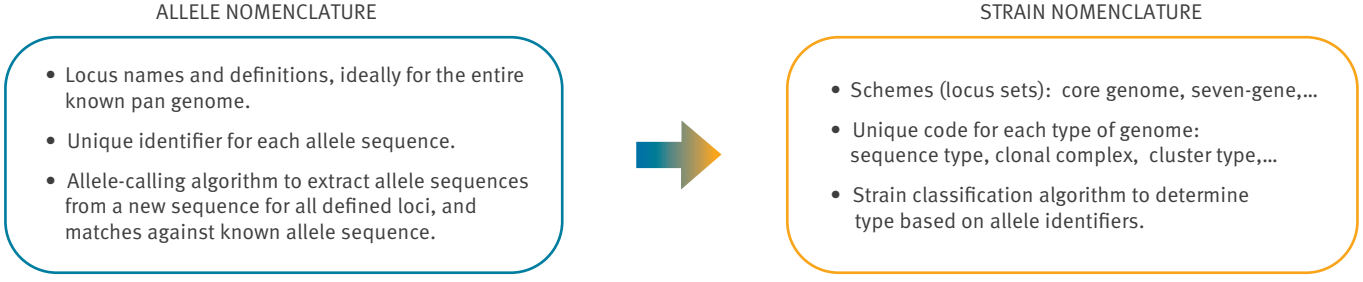
Allele nomenclature provides the names and definitions of all loci included in the wg/cgMLST scheme, as well as correspondence between allele sequences and allele identifiers (Figure 3).

The latter consists of unique identifiers, integers, that each correspond to a particular allele sequence for a pre-defined locus on the genome, and which do not change over time. Assignment of allele identifiers converts the WGS data for an isolate into a series of identifiers, one per locus. The resulting allelic profiles are used to determine genetic similarity between isolates. For PulseNet International laboratories to maintain real-time outbreak detection and inter-laboratory comparability, it is crucial that allele calling can be done reliably in real-time; i.e. automated, and that the nomenclature scheme used is the same in all laboratories. This will ultimately require a global allele-calling algorithm, which is still in the early stages. Issues to be addressed are the use of sequence reads vs assemblies, assembly method, locus start and end definitions, parameters for locus identification and allele sequence extraction, algorithm accuracy per locus, etc.; the amount of manual curation may initially be high but ultimately the actual allele calling will be fully automatic. The wg/cgMLST approach will also necessitate an international database of alleles; there are several features of such a database that would be highly beneficial to international public health users (Table 2).

Organisations producing WGS data have differing policies on data sharing, so a global allele nomenclature database that strives to meet the needs of most is optimal. For example, it must be possible to submit not only whole genomes as raw reads or assembly, but also individual new alleles detected through the use of a local cached allele nomenclature database. In case of full genomes, it must be possible to have them automatically deleted once alleles have been called unless the submitter wants the full sequence to become available to the general public and/or the allele database curators. Because of the substantial size of raw read data, an allele calling platform would also need sufficient bandwidth to support such large amounts of data being continuously uploaded by organisations worldwide, along with computational resources available to process alleles and return results in real-time. A flexible system that has an open interface would facilitate integration with existing organisations' or other third party systems. Quality control must be carefully planned as part of allele calling, as poor quality or dubious alleles, or alleles that match several paralogous genes, should not be used as reference alleles due to the reduction in reproducibility and deterioration of quality. For all

Figure 3

Allele and strain nomenclature



submissions, the provision to the user of an automated quality assessment is essential. The selection of additional reference alleles will require manual intervention by curators, but this is expected to be infrequent. New loci should not routinely be added to an existing wgMLST scheme since this invariably will also require revision of the strain nomenclature database (see next section). Security features would mitigate inadvertent or malicious use (e.g. submissions of large amounts of artificial sequences) while ensuring that individual allele sequences and their identifiers remain publicly accessible – perhaps by requiring user registration for submissions. Finally, a process to solicit input from stakeholders and the development of a collaboration agreement or terms of service would facilitate use by many public health organisations. Until the global allele nomenclature database has been established, PulseNet International members have informally agreed to work with local or regional versions of the same extended MLST scheme and use internally the same allele calling method for each pathogen.

Strain nomenclature

Strain nomenclature is a construct devised to classify and accordingly label an isolate, placing it into a designated category within the diversity of the species. It is essential in food-borne disease outbreak investigations for simplified and rapid communication among stakeholders. These include epidemiologists, medical and food safety officers, inspectors, and healthcare and environmental health professionals, wherein rapid and effective communication is best achieved through simple tables, line lists, outbreak reports, and person-to-person conversations, without the need to explain the topology of a phylogenetic tree. A strain nomenclature derived from allele identifiers but also reflecting phylogeny (and epidemiologically relevant) would be ideal. Such nomenclatures are hierarchical when they consist of codes that contain several levels of similarity [21,22]. When two isolates share only part of the code – e.g. '1.2.3' vs '1.2.4' – they are similar to some extent since they are both classified as '1.2' and more similar than isolates that differ in the second digit, e.g. '1.2.3' vs '1.4.9', which in turn are more similar than isolates that do not have the first digit in common, e.g. '1.2.3' vs '2.1.4'. The definition of a species' core genome, i.e. the loci common to nearly all isolates of a particular species (or lineage) is critical for the development of strain nomenclature. For some pathogens, whole genome or pangenome, i.e. all loci detected in any isolate of a species, may also be used for strain nomenclature. Core genomes have been suggested for *L. monocytogenes*, *C. jejuni/coli*, *S. enterica*, and *E. coli* (http://bigsdb.pasteur.fr/listeria/listeria.html; http://pubmlst.org/campylobacter; https://enterobase.warwick.ac.uk) [23,24]. The stability and accuracy of taxonomical nomenclature is not yet well established, although they are expected to be suitable for surveillance purposes. For those pathogens for which a hierarchical nomenclature may not be possible, categorical nomenclatures akin to the sequence types and clonal complexes for seven-gene MLST can always be derived. The algorithm for strain classification should ideally be stable, i.e. reproducibly return the same code for the same isolate on repeated testing. As is the case for the allele nomenclature database, an open interface for machine-to-machine communication and authentication of users should also be in place. It has much lower infrastructure requirements than allele nomenclature since only allele identifiers need to be submitted. Global strain nomenclature schemes should ideally be curated by the subject-matter experts who developed the underlying MLST schemes; this will require collaboration and cooperation between public health authorities and academic experts.

Data sharing

Rapid sharing of subtyping data between country or regional coordinators has been achieved in PulseNet International to-date by the exchange of PFGE data files on a members-only Internet-based discussion board on an as-needed basis. Typically, this occurs

Table 2

General requirements for a global allele nomenclature database, May 2017

ID	Description
1	Submission of sequence data and any subsequent allele calling can only be done by and for registered users. The nomenclature database content on the other hand, including the full set of known unique allele sequences and their identifiers, must be publicly accessible.
2	All nomenclature related functionality of the database must be free of charge to end users. Long-term sustainability and portability to other servers must be addressed.
3	The database must have close to 100% guaranteed uptime and have sufficient bandwidth to support upload of data by organisations worldwide. Sufficient computing power must be available to perform quality controls and allele calling in real time.
4	It must be possible to submit either raw reads or individual allele sequences, in order to retrieve the corresponding allele identifiers as well as any quality control results. Raw reads or any derived data other than individual new alleles may not be stored permanently in the database or used for any other purpose than deriving allele nomenclature. If needed for practical reasons, submission of raw reads can be implemented at a later stage.
5	There must be an open interface for machine-to-machine communication that covers all of the publicly available functionality. A formal process to incorporate input and agreement from stakeholders on changes to the system must be in place.
6	It must be possible for authorised curator users to annotate individual allele sequences with information, e.g. to include them as a reference allele, and to add additional loci to derive allele nomenclature for, as the known pan genome grows.

after a potential outbreak has already been flagged by one country/region, with the exception of the US and Canada who permit direct access to each other's national databases. National or regional databases are accessed directly by internal network members. With the limited utility of PFGE data outside of outbreak detection and response, there has not been cause to make these data more widely available on a routine basis. Also, sharing of real-time PFGE results outside the network has historically been strictly controlled, executed according to agreements negotiated by coordinating country/regional laboratories and their submitters (typically state, provincial, or local laboratories) whose individual privacy legislations stipulate conditions for sharing.

However, genome sequences can be used for many more purposes than routine public health surveillance and outbreak response, in particular they are highly suitable for basic research and activities aligned with the One Health approach [25]. Ideally, the genome sequences collected for surveillance purposes should be publicly available, if not in real time then within a reasonable time frame, e.g. within 12 months of sequence generation. These data should comprise both the sequences and some descriptive data about the isolate, as the sequences themselves alone have extremely limited utility. Publicly available repositories within the INSDC accessible via the Internet would be the ideal storage location, i.e. the SRA, the European Nt Archive and the DNA Data Bank of Japan as these are synchronised with each other on a regular basis. Each organisation or country/region determines itself which sequence data to upload, along with what descriptive data (e.g. time, place and type of isolate) would be uploaded to these repositories. Current data sharing procedures in some PulseNet International participating laboratories show promise in providing a suitable balance between protecting the privacy of patients and the utility of data generated and shared in real-time for global surveillance; these procedures include providing very little descriptive data for each isolate initially followed by the addition of information about the isolate after a 6–12 month delay.

At present, not all public sequence repositories allow for user-defined controls (e.g. sharing WGS data with a specific user subset immediately after upload and during a period of embargo before public release); however, such functionality as well as an improved interface may accelerate implementation of WGS and data sharing by more organisations. Given the expertise, infrastructure, mission and stability of the public repositories, integrating the global allele nomenclature databases, the allele-calling algorithms and possibly the strain nomenclature databases into these public spheres with an appropriate curator interface for subject matter experts would be desirable at the global level. Storing the allele identifiers along with the WGS data in the sequence repository would effectively make the latter searchable for the presence of loci, specific alleles and specific mutations. Since the principle of allele nomenclature is species independent, it could even be applied to all species rather than only those relevant for food- and waterborne diseases, to the benefit of all users of these databases.

Validation

Like other subtyping methods, WGS-based tests must be thoroughly validated before implementation in routine public health practice. For a global network like PulseNet International, validation ensures that the method is applicable for strains encountered by all network members, performance is suitable, and it also generates interpretive guidelines necessary for the consistent interpretation of WGS results. Methods are subject to three phases of validation: internal, external (see below for explanation), and post-implementation evaluation and refinement. Ideally, all phases should be completed before implementation network-wide. However, the rapid demonstration of the superior technical performance of WGS compared with methods previously accepted as gold standards (e.g. PFGE, MLVA) has accelerated its implementation in many laboratories, before the formal completion of validation at the international level. Regardless of current individual country status, validation is ultimately necessary to ensure evidence standards for public health decision making can be consistently met.

Internal validation

The purpose of internal validation is to verify the robustness and technical performance of the method using a well-defined set of isolates. For internal validation of wg/cgMLST-based approaches, for example, this includes an assessment of each locus plus the scheme as a whole for reproducibility, stability, discrimination and epidemiological concordance, and a comparison with current gold standards.

External validation and post-implementation evaluation

During external validation, the portability and inter-laboratory robustness of the method are tested on a common isolate set in a wider number of laboratories. Typically this includes geographically dispersed laboratories with different levels of expertise and access to a wide variety of equipment and reagents. The external validation should also include prospective (or retrospective, if necessary) testing of isolate sets collected from each laboratory's routine operations. Once implemented, periodic evaluations are conducted for continual improvement. This is done to detect problems not identified during the initial validation, to assess the impact of any proposed changes to laboratory equipment and reagents, and to accommodate new bioinformatics methods. With the fields of genomics and bioinformatics currently experiencing rapid rates of advances, international systems such as PulseNet should be flexible enough to allow improvements to the process while protecting the integrity of inter-laboratory comparability and quality control.

Quality control

While quality control for individual laboratories is important, it is critical for a network wherein the results generated by laboratories other than your own are relied upon to support local and multi-jurisdictional public health decision making. A rigorous quality control programme ensures that correct results are reliably produced by all participants at all times and provides checkpoints during the process to flag and remove potentially incorrect results.

Routine quality parameters

Quality of the raw reads must be assessed; if the minimum quality metrics are not met, the reads must be discarded and sequencing repeated. All platforms produce raw reads of both sequences and the individual quality scores. A primary quality metric is coverage, defined as the number of raw reads for the average read length over the genome (i.e. the number of times each base in the genome is contained in individual raw reads). A minimum coverage of 30x is typically enough for routine surveillance, but this is platform-dependent and also may vary by organism [21,26]. Other quality metrics to be checked routinely include the average Phred or Q scores as a measure of base-calling accuracy. Low quality reads should not be uploaded to a repository or PulseNet database, open or closed to the public. Taxonomy check should also be included in the basic raw read quality check since isolate mix-ups and mixed cultures are the most common errors encountered in the laboratories generating sequencing data. Similarly, routine quality parameters such as the proportion of core genome loci detected through allele calling should also be implemented for the bioinformatics pipeline(s) used for analysis.

Ensuring standardisation and competence

As basic tenets of quality control, written standard operating procedures must be in place for both sequencing and analysis and the procedures should only be performed by trained personnel. Participation in an external quality assessment (EQA) programme is recommended. While each country/region sets its own quality control requirements, many PulseNet International participating countries submit and pass an organism-specific certification test before they are permitted to submit data to a repository or to most PulseNet country/regional databases (although this is not the case in Europe or PulseNet Asia-Pacific). The certification process documents each participant's highest level of competence in producing raw sequence data, performing analysis using bioinformatics tools, and checking data quality (participants may be certified in producing raw sequence data, analysing the results, or both). Initial PulseNet certification is followed by annual proficiency tests to ensure that competence is maintained. These quality control procedures are largely based on the well-established PulseNet International quality control programme for molecular subtyping; however, they may be further modified as needed to best suit WGS, as part of the continual evaluation and refinement process.

Implementation

Global whole genome sequencing implementation is not all-or-nothing

The benefits of genome sequencing have driven its use for public health decision making despite the lack of fully developed systems and infrastructure [27]. Across members of PulseNet International, the maturity and complexity of internal country/regional laboratory surveillance networks, the availability of funding and human resources, as well as the relative importance of infections caused by unsafe food among all infectious diseases differ across countries, according to each country's own priorities and resources. Thus, the strategy and timing for implementation of WGS for food-borne disease surveillance will vary around the world. At the same time, the costs of WGS are often already lower than the currently used characterisation methods including PFGE and MLVA and will likely decrease further [21]. Recently, recommendations have been published to guide both developed and developing countries in determining their readiness for implementing WGS [28]. With readiness dependent on a wide variety of political, technical, economic and political factors; e.g. infrastructure, equipment, training, operating funds, policies, etc.; each country will set their own timeline for implementation. Some countries have been able to make rapid transitions to WGS (i.e. within 12 months or less), others have had a slower pace spanning multiple years. Internationally, a gradual transition from traditional molecular methods to genome sequencing would ensure that countries not able to immediately implement WGS are not separated from the rest of the public health community [21]. Waiting for universal readiness before moving forward is also not advised; this would delay the manifestation of WGS benefits to food-borne disease management and prevention overall. There is a risk of dividing countries according to genomics capacity, furthering health inequalities due to economic status and hampering efforts to reduce disease burden and further efforts towards One Health [25].

The role of PulseNet International's public health laboratory network

PulseNet International's extensive network of laboratories, and history of standardisation and sharing, uniquely position it to guide WGS implementation worldwide in a manner that minimises isolating individual countries or regions. As revolutionary as genomics is for food-borne disease (and virtually all areas of infectious disease public health), these challenges of international compatibility are not new. These very same issues were faced during the implementation of PFGE in PulseNet International starting two decades ago [2]. Global standardisation was achieved through the network's capacity building and training activities, consensus decision making among participants, and a

careful balance between scientific merit and practical issues, these strengths provide the foundation to build a new system based on WGS. The members of PulseNet International are almost exclusively focused on the needs of routine surveillance for outbreak detection and response, which enables advances in genomics and bioinformatics to be harnessed and tailored to those specific needs while simultaneously advocating for the availability of data for broader purposes. The sharing of experiences and 'lessons learned' is another key role of PulseNet International; routine communication and sharing of protocols, policies, implementation plans, etc., across the network enables all members to benefit from the experience of early adopters.

Other national and international initiatives and networks lead or contribute substantially to the development of genomics and bioinformatics in the area of food safety. For example, the GenomeTrakr network (US Food and Drug Administration) has pioneered open source tools, public data sharing, and the application of WGS to regulatory and compliance activities [29]. PulseNet International is the public health-focused network of laboratories responsible for surveillance and outbreak response, which is complementary to GenomeTrakr activities. In the European Union, the European Centre for Disease Prevention and Control and the European Food Safety Authority collaborate to collect and analyse molecular typing data in a single database, providing centralised surveillance capability. The Global Microbial Identifier initiative is the forerunner for planning global genomics systems for all infectious diseases in all sectors of science and beyond [7]. The European COMPARE project leverages INSDC infrastructure and is working to provide a platform to support WGS data sharing and analysis (http://www.compare-europe.eu/). Additional organisations also play pivotal roles in international food safety, including the World Health Organization of the United Nations' INFOSAN and Global Food-borne Infections Network, and the Food and Agricultural Organisation of the United Nations. These organisations are also leaders in capacity building and knowledge transfer for WGS and its impacts on food-borne disease surveillance, as well as for regulatory and policy framework guidance.

Beyond the laboratory

For the integration of WGS for routine surveillance, all public health-related professionals must be prepared for the use of genomic data to support public health decision making. WGS should not be portrayed as a panacea; it does not replace the need for high quality epidemiological data to be interpreted in context with laboratory results [30]. Knowledge transfer must occur not only for the participating public health laboratories, but also for the national and regional epidemiologists and other immediate stakeholders. PulseNet member laboratories often serve as the interface between food-borne disease laboratory and epidemiology, and between public health and food monitoring. Understanding basic genomics and bioinformatics concepts by all members of the food safety continuum is needed.

Conclusion

To meet the needs of real-time surveillance, PulseNet International will standardise WGS-based subtyping using extended MLST-based approaches; specifically, wgMLST. This delivers optimal resolution and epidemiological concordance while providing unambiguous nomenclature. In addition, it is computationally efficient and realistic for most public health laboratories to use on a daily basis. Standardised protocols, validation studies, quality control programmes, database and scheme development, and training materials all must support the implementation and decentralisation of any new technique. As was done previously with PFGE, PulseNet International is presently disseminating these important elements among network members. Training, in particular, is a keystone for the success of standardised PulseNet activities; this will require dedicated and sustainable resources. A recent economic evaluation of PulseNet activities suggests that in the US alone, PulseNet prevents at least 270,000 illnesses from *S. enterica*, *E. coli*, and *L. monocytogenes* and saves 500 million US dollars (447 million euros) every year in medical costs and lost productivity [31]. The economic return on investment in PulseNet is approximately 70 US dollars/euros of benefit for every 1 dollar/euro invested by public health agencies. This demonstrates a significant economic and public health benefit from the system, and these impacts are expected to be even greater with the superior performance of WGS.

In order to truly standardise food-borne disease subtyping across the world, a public WGS-based nomenclature, curated where necessary, must be available as WGS data are not only suitable for surveillance and outbreak purposes, but also for answering other scientific questions (e.g. source attribution, antimicrobial resistance, transmission patterns, population structure, etc.). The WGS data themselves (including a minimum set of descriptive data) should ideally also be publicly available. To fully realise this vision, technical and political challenges must be overcome. PulseNet International will leverage its experience to guide the implementation of genome sequencing in a manner that both meets immediate public health needs and supports broader efforts to preventing and mitigating the effects of food-borne disease worldwide.

FWD-NEXT Expert Panel Members

Ariane Pietzka (Österreichische Agentur für Gesundheit und Ernährungssicherheit, Graz, Austria); Eva Trost (Robert Koch-Institut, Wernigerode, Germany); Kristoffer Kiil (Statens Serum Institut, Copenhagen, Denmark); Ivelina Damjanova (Országos Epidemiológia Központ, Budapest, Hungary); Valeria Michelacci (Istituto Superiore di Sanità, Rome, Italy); Joël Mossong (Laboratoire National de Santé, Luxembourg); Eelco Franz (Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven, Netherlands); Wilfrid van Pelt (Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven, Netherlands); Tomasz Wołkowicz (National Institute of Public Health – National Institute of Hygiene, Warsaw, Poland); Vítor Borges (Instituto

Nacional de Saúde Doutor Ricardo Jorge, Lisbon, Portugal); Cecilia Jernberg (Folkhälsomyndigheten, Stockholm, Sweden); Erik Alm (Folkhälsomyndigheten, Stockholm, Sweden); Ian Fisher (Public Health England, London, United Kingdom); Kathie Grant (Public Health England, London, United Kingdom); Tim Dallman (Public Health England, London, United Kingdom); Joakim Ågren (Statens veterinärmedicinska anstalt, Uppsala, Sweden); Martin Maiden (University of Oxford, Oxford, United Kingdom); Sylvain Brisse (Institut Pasteur, Paris, France); João André Carriço (Universidade de Lisboa, Lisbon, Portugal); Guy Cochrane (European Molecular Biology Laboratory, European Bioinformatics Institute, Hinxton, United Kingdom);
Beatriz Guerra (European Food Safety Authority, Parma, Italy); Maria Teresa Da Silva Felicio (European Food Safety Authority, Parma, Italy); Daniel Palm (European Centre for Disease Prevention and Control); Joana Revez (European Centre for Disease prevention and Control); Marc Struelens (European Centre for Disease Prevention and Control).

Acknowledgments

Acknowledgements for manuscript review and feedback: Aleisha Reimer (Public Health Agency of Canada); Natalie Knox (Public Health Agency of Canada); Jennifer Adams (Association of Public Health Laboratories, USA).
The views and opinions of the authors expressed herein do not necessarily state or reflect those of the Centers for Disease Control and Prevention, the European Centre for Disease Prevention and Control or the European Food Safety Authority .
Use of trade names is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention or by the US Department of Health and Human Services.

Conflict of interest

None declared.

Authors' contributions

Celine Nadon, Lead author; wrote sections, conception and design, critical review and intellectual input; Ivo Van Walle, Lead author, coordination and drafting of nomenclature, conception and design, critical review and intellectual input (Celine Nadon and Ivo Van Walle contributed equally); Peter Gerner-Smidt, Wrote sections, conception and design, critical review and intellectual input; Josefina Campos, Wrote sections, conception and design, critical review and intellectual input; Isabel Chinen, Wrote sections, conception and design, critical review and intellectual input; Jeniffer Concepcion Acevedo, Critical review and intellectual input, citation research and wrote reference section; Brent Gilpin, Drafted figures, conception and design, critical review and intellectual input; Anthony M. Smith, Conception and design, critical review and intellectual input; Kai Man Kam, Conception and design, critical review and intellectual input; Enrique Perez, Conception and design, critical review and intellectual input; Eija Trees, Wrote sections, conception and design, critical review and intellectual input; Kristy Kubota, Critical review and intellectual input; Johanna Takkinen, critical review and intellectual input; Eva Møller Nielsen, Critical review and intellectual input; Heather Carleton, Wrote sections, conception and design, critical review and intellectual input; FWD-NEXT Expert Panel, Critical input and review of nomenclature section.

References

1. World Health Organization (WHO). WHO estimates of the global burden of foodborne diseases. Technical report. Geneva: WHO. [Accessed 16 Dec 2016]. Available from: http://www.who.int/foodsafety/publications/foodborne_disease/fergreport/en/
2. Swaminathan B, Gerner-Smidt P, Ng L-K, Lukinmaa S, Kam K-M, Rolando S, et al. Building PulseNet International: an interconnected system of laboratory networks to facilitate timely public health recognition and response to foodborne disease outbreaks and emerging foodborne diseases. Foodborne Pathog Dis. 2006;3(1):36-50. http://dx.doi.org/10.1089/fpd.2006.3.36 PMID:16602978
3. Nadon CA, Trees E, Ng LK, Møller Nielsen E, Reimer A, Maxwell N, et al. Development and application of MLVA methods as a tool for inter-laboratory surveillance. Euro Surveill. 2013;18(35):20565. http://dx.doi.org/10.2807/1560-7917.ES2013.18.35.20565 PMID:24008231
4. Barrett TJ, Gerner-Smidt P, Swaminathan B. Interpretation of pulsed-field gel electrophoresis patterns in foodborne disease investigations and surveillance. Foodborne Pathog Dis. 2006;3(1):20-31. http://dx.doi.org/10.1089/fpd.2006.3.20 PMID:16602976
5. Rumore JL, Tschetter L, Nadon C. The Impact of Multilocus Variable-Number Tandem-Repeat Analysis on PulseNet Canada Escherichia coli O157:H7 Laboratory Surveillance and Outbreak Support, 2008-2012. Foodborne Pathog Dis. 2016;13(5):255-61. http://dx.doi.org/10.1089/fpd.2015.2066 PMID:26990274
6. Pichel M, Brengi SP, Cooper KLF, Ribot EM, Al-Busaidy S, Araya P, et al. Standardization and international multicenter validation of a PulseNet pulsed-field gel electrophoresis protocol for subtyping Shigella flexneri isolates. Foodborne Pathog Dis. 2012;9(5):418-24. http://dx.doi.org/10.1089/fpd.2011.1067 PMID:22506731
7. Aarestrup FM, Brown EW, Detter C, Gerner-Smidt P, Gilmour MW, Harmsen D, et al. Integrating genome-based informatics to modernize global disease monitoring, information sharing, and response. Emerg Infect Dis. 2012;18(11):e1. http://dx.doi.org/10.3201/eid1811.120453 PMID:23092707
8. Carleton HA, Gerner-smidt P. Whole-Genome Sequencing Is Taking over Foodborne Disease Surveillance. Microbe Mag. 2016;11(7):311-7. Available from: http://www.asmscience.org/content/journal/microbe/10.1128/microbe.11.311.1.
9. Dallman T, Inns T, Jombart T, Ashton P, Loman N, Chatt C, et al. Phylogenetic structure of European Salmonella Enteritidis outbreak correlates with national and international egg distribution network. Microb Genom. 2016;2(8):e000070. http://dx.doi.org/10.1099/mgen.0.000070 PMID:28348865
10. Jackson BR, Tarr C, Strain E, Jackson KA, Conrad A, Carleton H, et al. Implementation of Nationwide Real-time Whole-genome Sequencing to Enhance Listeriosis Outbreak Detection and Investigation. Clin Infect Dis. 2016;63(3):380-6. http://dx.doi.org/10.1093/cid/ciw242 PMID:27090985
11. Holmes A, Allison L, Ward M, Dallman TJ, Clark R, Fawkes A, et al. Utility of whole-genome sequencing of Escherichia coli O157 for outbreak detection and epidemiological surveillance. J Clin Microbiol. 2015;53(11):3565-73. http://dx.doi.org/10.1128/JCM.01066-15 PMID:26354815
12. Mossong J, Decruyenaere F, Moris G, Ragimbeau C, Olinger CM, Johler S, et al. Investigation of a staphylococcal food poisoning outbreak combining case-control, traditional typing and whole genome sequencing methods, Luxembourg, June 2014. Euro Surveill. 2015;20(45):30059. http://dx.doi.org/10.2807/1560-7917.ES.2015.20.45.30059 PMID:26608881
13. Butcher H, Elson R, Chattaway MA, Featherstone CA, Willis C, Jorgensen F, et al. Whole genome sequencing improved case ascertainment in an outbreak of Shiga toxin-producing Escherichia coli O157 associated with raw drinking milk. Epidemiol Infect. 2016;144(13):2812-23. http://dx.doi.org/10.1017/S0950268816000509 PMID:27338677
14. Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, et al. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic Escherichia coli. J Clin Microbiol. 2014;52(5):1501-10. http://dx.doi.org/10.1128/JCM.03617-13 PMID:24574290
15. The INFOSAN Activity Report 2014/2015. Geneva: World Health Organization. [Accessed 16 Dec 2016]. Available from: http://www.who.int/foodsafety/publications/infosan_activity2014-15/en/
16. Mardis ER. Next-generation sequencing platforms. Annu Rev Anal Chem (Palo Alto, Calif). 2013;6(1):287-303. http://dx.doi.org/10.1146/annurev-anchem-062012-092628 PMID:23560931
17. Xavier BB, Sabirova J, Pieter M, Hernalsteens J-P, de Greve H, Goossens H, et al. Employing whole genome mapping for optimal de novo assembly of bacterial genomes. BMC Res Notes. 2014;7(1):484. http://dx.doi.org/10.1186/1756-0500-7-484 PMID:25077983

18. Gardner SN, Hall BG. When whole-genome alignments just won't work: kSNP v2 software for alignment-free SNP discovery and phylogenetics of hundreds of microbial genomes. PLoS One. 2013;8(12):e81760. http://dx.doi.org/10.1371/journal.pone.0081760 PMID:24349125
19. Maiden MC, Jansen van Rensburg MJ, Bray JE, Earle SG, Ford SA, Jolley KA, et al. MLST revisited: the gene-by-gene approach to bacterial genomics. Nat Rev Microbiol. 2013;11(10):728-36. http://dx.doi.org/10.1038/nrmicro3093 PMID:23979428
20. Wasyl D, El Sedawy A, Lukinmaa S. PulseNet Europe - international molecular subtyping network for food-borne disease surveillance. Med Weter. 2008;64(2):123-6.
21. European Centre for Disease Control and Prevention (ECDC). Expert Opinion on the introduction of next-generation typing methods for food- and waterborne diseases in the EU and EEA Stockholm: ECDC; 2014. [Accessed Dec 2016]. Available from: http://ecdc.europa.eu/en/publications/Publications/food-and-waterborne-diseases-next-generation-typing-methods.pdf
22. Marakeby H, Badr E, Torkey H, Song Y, Leman S, Monteil CL, et al. A system to automatically classify and name any individual genome-sequenced organism independently of current biological classification and nomenclature. PLoS One. 2014;9(2):e89142. http://dx.doi.org/10.1371/journal.pone.0089142 PMID:24586551
23. Moura A, Criscuolo A, Pouseele H, Maury MM, Leclercq A, Tarr C, et al. Whole genome-based population biology and epidemiological surveillance of Listeria monocytogenes. Nat Microbiol. 2016;2:16185. http://dx.doi.org/10.1038/nmicrobiol.2016.185 PMID:27723724
24. Sheppard SK, Jolley KA, Maiden MC. A Gene-By-Gene Approach to Bacterial Population Genomics: Whole Genome MLST of Campylobacter. Genes (Basel). 2012;3(2):261-77. http://dx.doi.org/10.3390/genes3020261 PMID:24704917
25. World Health Organization (WHO). One Health. Geneva: WHO; April 2017. Available from: http://www.who.int/features/qa/one-health/en/
26. Shendure J, Ji H. Next-generation DNA sequencing. Nat Biotechnol. 2008;26(10):1135-45. http://dx.doi.org/10.1038/nbt1486 PMID:18846087
27. Olsen RJ, Long SW, Musser JM. Bacterial genomics in infectious disease and the clinical pathology laboratory. Arch Pathol Lab Med. 2012;136(11):1414-22. http://dx.doi.org/10.5858/arpa.2012-0025-RA PMID:22439809
28. Food and Agriculture Organization (FAO). Application of whole genome sequencing in food safety management. Rome: FAO; 2016. [Accessed 28 Aug 2016]. Available from: http://www.fao.org/documents/card/en/c/61e44b34-b328-4239-b59c-a9e926e327b4/.
29. Allard MW, Strain E, Melka D, Bunning K, Musser SM, Brown EW, et al. Practical Value of Food Pathogen Traceability through Building a Whole-Genome Sequencing Network and Database. J Clin Microbiol. 2016;54(8):1975-83. http://dx.doi.org/10.1128/JCM.00081-16 PMID:27008877
30. Stasiewicz MJ, Oliver HF, Wiedmann M, den Bakker HC. Whole-Genome Sequencing Allows for Improved Identification of Persistent Listeria monocytogenes in Food-Associated Environments. Appl Environ Microbiol. 2015;81(17):6024-37. http://dx.doi.org/10.1128/AEM.01049-15 PMID:26116683
31. Scharff RL, Besser J, Sharp DJ, Jones TF, Peter G-S, Hedberg CW. An Economic Evaluation of PulseNet: A Network for Foodborne Disease Surveillance. Am J Prev Med. 2016;50(5) Suppl 1;S66-73. http://dx.doi.org/10.1016/j.amepre.2015.09.018 PMID:26993535

License and copyright

Research article

Determinants of success and sustainability of the WHO multimodal hand hygiene promotion campaign, Italy, 2007–2008 and 2014

ML Moro [1], F Morsillo [1], S Nascetti [2], M Parenti [3], B Allegranzi [4], MG Pompa [5], D Pittet [6]

1. Agenzia Sanitaria e Sociale Regione Emilia-Romagna, Bologna, Italy
2. Dipartimento di Sanità Pubblica, Area Igiene e Sanità Pubblica, Azienda USL, Bologna, Italy
3. Azienda Ospedaliero-Universitaria di Bologna Policlinico S.Orsola-Malpighi, Bologna, Italy
4. Infection Prevention and Control Global Unit, WHO Service Delivery and Safety, World Health Organization, Geneva, Switzerland
5. Ufficio 5 - Rapporti con l'Unione Europea, il Consiglio d'Europa, l'OCSE, l'OMS, e le altre agenzie ONU ed Organizzazioni internazionali, Direzione generale della comunicazione e dei rapporti europei e internazionali, Ministero della Salute, Rome, Italy
6. Infection Control Programme and WHO Collaborating Centre on Patient Safety, University of Geneva Hospitals and Faculty of Medicine, Geneva, Switzerland

Correspondence: Filomena Morsillo (fmorsillo@regione.emilia-romagna.it)

Citation style for this article:
Moro ML, Morsillo F, Nascetti S, Parenti M, Allegranzi B, Pompa MG, Pittet D. Determinants of success and sustainability of the WHO multimodal hand hygiene promotion campaign, Italy, 2007–2008 and 2014. Euro Surveill. 2017;22(23):pii=30546. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2017.22.23.30546

Article submitted on 30 June 2016 / accepted on 28 April 2017 / published on 08 June 2017

A national hand hygiene promotion campaign based on the World Health Organization (WHO) multimodal, Clean Care is Safer Care campaign was launched in Italy in 2007. One hundred seventy-five hospitals from 14 of 20 Italian regions participated. Data were collected using methods and tools provided by the WHO campaign, translated into Italian. Hand hygiene compliance, ward infrastructure, and healthcare workers' knowledge and perception of healthcare-associated infections and hand hygiene were evaluated before and after campaign implementation. Compliance data from the 65 hospitals returning complete data for all implementation tools were analysed using a multilevel approach. Overall, hand hygiene compliance increased in the 65 hospitals from 40% to 63% (absolute increase: 23%, 95% confidence interval: 22–24%). A wide variation in hand hygiene compliance among wards was observed; inter-ward variability significantly decreased after campaign implementation and the level of perception was the only item associated with this. Long-term sustainability in 48 of these 65 hospitals was assessed in 2014 using the WHO Hand Hygiene Self-Assessment Framework tool. Of the 48 hospitals, 44 scored in the advanced/intermediate categories of hand hygiene implementation progress. The median hand hygiene compliance achieved at the end of the 2007–2008 campaign appeared to be sustained in 2014.

Introduction

In recent years, increasing attention has been given to hand hygiene as a leading measure to prevent the spread of antimicrobial resistance and to reduce healthcare-associated infections (HAIs) [1]. Several studies offer convincing evidence that improved hand hygiene practices lead to a reduction of HAIs and/or transmission or colonisation by multidrug-resistant organisms (MDROs) [2].

In October 2005, the World Health Organization (WHO) launched Clean Care is Safer Care, a global hand hygiene campaign [3]. Aimed to reduce HAIs it focused on implementing new hand hygiene recommendations [4] through a multimodal hand hygiene promotion campaign [5]. At that time, the Italian Minister of Health signed a statement to show its commitment to reducing HAIs [6]. Consequently, a national hand hygiene campaign based on the materials provided by WHO was launched in November 2006. The campaign was organised by the national coordinating centre for HAIs (Agenzia Sanitaria e Sociale Regionale Emilia-Romagna) and funded by the National Centre for Disease Control (Centro Nazionale per la Prevenzione e il Controllo delle Malattie). The multimodal national campaign was conducted in 2007–2008 and not repeated in the following years.

This study reports the campaign's effect on hand hygiene compliance immediately after implementation, and identifies factors associated with the observed improvement at the individual level and at the ward level. It also reports on the level of hand hygiene compliance 7 years later.

Table 1

Compliance with hand hygiene at baseline and follow-up across professional categories, type of indication and ward, national campaign, Italy, 2007–2008

	Baseline		Follow-up			
	Opportunities (n)	Compliance (%)	Opportunities (n)	Compliance (%)	Absolute difference in compliance (%)	95% CI
Overall	18,045	40	17,577	63	+23	22–24
Professional category						
Nurses	11,732	42	11,506	67	+25	24–26
Medical doctors	3,849	39	3,693	55	+16	14–18
Auxiliary	1,960	33	2,114	61	+28	25–31
Other	504	28	264	46	+18	11–25
Hand hygiene indication						
Before patient contact	5,538	33	5,494	59	+26	24–28
Before aseptic task	2,109	45	2,008	64	+19	16–22
After contact with patient surroundings	3,602	25	3,141	50	+25	23–27
After patient contact	5,117	50	5,070	71	+21	19–23
After body fluid exposure risk	1,679	55	1,864	75	+19	16–22
Type of ward						
Surgical	4,762	31	4,735	56	+25	23–27
Intensive care	10,618	42	10,076	65	+23	22–24
Medical and other wards	2,665	45	2,766	67	+22	20–25
Type of hospital						
Private	599	25	589	45	+20	15–26
Public	15,511	40	15,190	63	+23	22–24
Research/teaching	1,935	41	1,798	71	+30	27–33

CI: confidence interval.

Methods

Implementing WHO's Clean Care is Safer Care campaign

In November 2006, Italian public hospitals were invited by regional coordinators to implement the WHO's Clean Care is Safer Care hand hygiene promotion campaign. Hospitals were asked to have at least one or two wards with at least one hand washing basin for every 10 beds participate, with intensive care units (ICUs), surgical wards or onco-haematology/transplant wards being most preferred.

The Italian campaign was based entirely on the WHO's [3], and involved all WHO documents and tools [7] being translated into Italian and then made available on the Ministry of Health website [8]. The campaign tools were focused on the following five elements: (i) system change, including access to alcohol-based hand rub (ABHR); (ii) healthcare workers' (HCWs) training and education; (iii) monitoring and feedback on practices; (iv) visual reminders in the workplace; and (v) institutional patient-safety climate [5,9].

Implementation of the campaign occurred from November 2006 onwards and in the following four stages: (i) preparedness (3 months on average); (ii) baseline evaluation (2.5 months on average); (iii) intervention (3 months on average); and (iv) follow-up evaluation (2.5 months on average) [5].

Evaluating short-term impact of the campaign

The follow-up evaluation (implementation stage 4 as per above) was carried out from March 2007 to October 2008. Only hospitals/wards with data available from both the baseline evaluation and follow-up evaluation phases were included in the analysis.

Participating hospitals were requested to send all data collected via four questionnaires, provided by the WHO campaign [5,7] and translated into Italian [8], for facility situation (at baseline only), ward infrastructure, hand hygiene knowledge (anonymous) and hand hygiene perception (anonymous), as well as observations about hand hygiene compliance to the Agenzia Sanitaria e Sociale Regionale Emilia-Romagna. Hand hygiene perception explored HCW's perceptions about their own hand hygiene compliance, the compliance of other HCWs, the impact of HAIs, the importance of

Table 2

Factors associated with hand hygiene compliance at baseline and follow-up, national campaign, Italy, 2007–2008

	Baseline			Follow-up		
	OR	95% CI	p value	OR	95% CI	p value
Hand hygiene indication						
After body fluid exposure risk	Ref.	NA	NA	Ref.	NA	NA
After patient contact	0.75	0.65–0.85	< 0.0001	0.78	0.68–0.89	< 0.0001
After contact with patient surroundings	0.28	0.24–0.32	<0.0001	0.32	0.28–0.37	<0.0001
Before patient contact	0.32	0.28–0.37	0.004	0.45	0.39–0.52	<0.0001
Before aseptic task	0.59	0.50–0.68	<0.0001	0.54	0.46–0.64	<0.0001
Professional category						
Nurses	Ref.	NA	NA	Ref.	NA	NA
Medical doctors	0.74	0.68–0.81	<0.0001	0.54	0.49–0.59	<0.0001
Auxiliary	0.69	0.61–0.79	<0.0001	0.81	0.72–0.92	<0.0001
Other professionals	0.48	0.38–0.61	<0.0001	0.31	0.23–0.42	<0.0001
Type of hospital						
Research/teaching	Ref.	NA	NA	Ref.	NA	NA
Private	1.39	0.26–2.86	0.643	1.00	0.24–2.41	0.995
Public	2.00	0.50–3.56	0.080	1.10	0.10–3.04	0.801
Facility situation score greater than the median[a]	1.12	0.09–3.03	0.645	1.35	0.13–3.49	0.211
Type of ward						
Medical and other wards	Ref.	NA	NA	Ref.	NA	NA
Surgical ward	0.48	0.35–0.85	0.047	0.69	0.66–1.92	0.296
ICU	1.08	0.54–1.85	0.818	0.91	0.78–3.13	0.770
Ward infrastructure for hand hygiene score greater than the median[a]	2.05	1.50–7.06	0.015	1.50	0.70–12.40	0.093
Knowledge score greater than the median[a]	1.42	0.19–5.65	0.144	1.28	0.24–6.76	0.313
Perception score greater than the median[a]	0.88	0.07–1.35	0.637	1.75	1.50–32.50	0.022
Measures of hand hygiene compliance variability						
Ward level variance[b]	0.92	0.69–1.37	NA	0.85	0.63–1.27	NA
Change in variance[c]	-22%	NA	NA	-30%	NA	NA
Intra-class correlation[d]	22%	NA	NA	20%	NA	NA

CI: confidence interval; ICU: intensive care unit; OR: odds ratio.

[a] Dummy variable yes vs no.

[b] Standard Error (SE) in the empty model is 1.182 (0.203) and 1.213 (0.216), baseline and follow-up respectively.

[c] Proportional reduction change in variance (PVC).

[d] Intra-class correlation coefficient (ICC) in the empty model is 26% and 29%, baseline and follow-up respectively.

hand hygiene as a preventive measure to reduce HAIs and the effectiveness of the different elements of a multimodal strategy. A total score for each of the four questionnaire areas [5,7] was calculated as the ratio of the response score to the maximum expected score (i.e. sum of the all items in the questionnaire/(overall maximum expected score × number of non-missing items)). Thus, each of the areas received scores ranging from 0 to 1.

Data on hand hygiene compliance was collected via a trained, unobtrusive observer who, during 20-minute sessions, openly observed staff and recorded the total number of hand hygiene opportunities and actions, either hand washing or hand rubbing [5,7]. An opportunity for hand hygiene was defined as the occurrence of any indication for hand hygiene according to the WHO 'My 5 Moments' approach [7,9,10]. Each ward had to record at least 200 opportunities; otherwise they were considered to have incomplete data and were excluded from the analysis [11].

Determining long-term sustainability of the 2007–2008 campaign in 2014

The long-term sustainability of hand hygiene behaviour change was assessed 7 years after the conclusion of the national campaign. In 2014, the 65 hospitals included in the follow-up evaluation of the 2007–2008 campaign were invited to complete the Hand Hygiene Self-Assessment Framework (HHSAF). This tool is part of the WHO Clean Care is Safer Care kit [12,13], but was not a part of the tools used in the baseline and follow-up evaluations. The questionnaire comprises 27 items grouped into five sections reflecting the five elements

Table 3

Survey results of hospitals participating in the 2007–2008 national campaign according to 2014 hand hygiene implementation level, Italy, 2014 (n = 48 hospitals)

	Implementation level in 2014			
	Advanced (n = 12)		Intermediate/basic (n = 36)	
2007–2008 campaign	**Median**	**Interquartile range**	**Median**	**Interquartile range**
Hand hygiene compliance (observed/expected)				
at baseline	0.52	0.22[a]	0.37	0.28[a]
at follow-up	0.74	0.21	0.63	0.28
Ward infrastructure for hand hygiene score (questionnaire)				
at baseline	0.67	0.25[a]	0.33	0.50[a]
at follow-up	0.84	0	0.83	0.17
Knowledge score (questionnaire)				
at baseline	0.53	0.07	0.53	0.07
at follow-up	0.75	0.24	0.74	0.26
Perception score (questionnaire)				
at baseline	0.77	0.11[a]	0.69	0.06[a]
at follow-up	0.84	0.08	0.77	0.07
2014 survey	**Median score/max achievable score**	**Interquartile range**	**Median score/max achievable score**	**Interquartile range**
HHSAF	450/500	60	322.5/500	77.5
Components scores of HHSAF				
System change	100/100	0	90/100	20
Training and education	100/100	10	65/100	10
Evaluation and feedback	80/100	10	55/100	35
Reminders in the workplace	90/100	20	65/100	12
Institutional safety climate for hand hygiene	90/100	30	40/100	20
Selected items of HHSAF				
Training and education[b]	40/40	0	20/40	20
Evaluation and feedback: hand hygiene compliance[c]	25/30	6	15/30	15
Institutional safety climate for hand hygiene[d]	20/20	0	15/20	11.2
Institutional safety climate for hand hygiene[e]	10/10	10	0/10	0
Institutional safety climate for hand hygiene[f]	5/5	0	0/10	5
Institutional safety climate for hand hygiene[g]	10/10	2.5	0/10	0

HHSAF: Hand Hygiene Self-Assessment Framework; IQR: interquartile range.

[a] Advanced vs intermediate/basic: p value < 0.05.

[b] Item 2.1a, 2.1b: Mandatory training for all professional categories at commencement of employment, then ongoing regular training (at least annually).

[c] Overall hand hygiene compliance rate according to the WHO Hand Hygiene Observation tool (or similar technique): 25 points corresponds to compliance equal to 71–80%; 15 points corresponds to compliance of 51–60%.

[d] Visible commitment to support hand hygiene improvement by the Chief Executive Officer, the Medical Director, the Director of Nursing.

[e] A clear plan for the promotion of hand hygiene throughout the entire facility for the annual global campaign on 5 May, Save Lives: Clean Your Hands.

[f] Patients informed about the importance of hand hygiene (e.g. via a leaflet).

[g] A formalised programme of patient engagement.

of the multimodal campaign (e.g. system change; training and education; evaluation and feedback; reminders in the workplace; and institutional safety climate). Each component section is scored out of 100 points (total maximum score: 500), and based on this, responding healthcare facilities were classified as inadequate (≤ 125 points), basic (126–250), intermediate (251–375) or advanced (› 375) [12,13]. Notably, the HHSAF asked hospitals to report on the level of hand hygiene compliance obtained through direct observation.

Statistical analysis

The following statistical methods were used to analyse the impact of the campaign.

Pearson's chi-squared test and McNemar test were used where appropriate to investigate the difference between proportions.

For facility situation, ward infrastructure, hand hygiene knowledge and hand hygiene perception, the

Cronbach's alpha coefficient was used to estimate the reliability of sets of questionnaire items before calculating the scores (alpha values higher than 70% were considered acceptable) and the internal consistency between different items [14]. A non-parametric K-sample test on the equality of medians was used to test the differences among scores.

Compliance data were analysed using a multilevel approach [15,16] with hand hygiene opportunities as first level and ward characteristics as second level. The following first level covariates were used: professional category, hand hygiene indication and study phase (baseline and follow-up). Second level covariates were ward specialty, type of facility, the score related to the facility situation before the intervention, the ward structure score for hand hygiene, and HCWs' knowledge and perception score in each ward before and after the campaign implementation. The number of observed opportunities per hour of observation [17,18] and the average hand hygiene compliance at baseline were used in the model evaluating the impact of the campaign.

A logistic multilevel regression model at mixed effects with binomial distribution and logit link was used to explore the effect of hand hygiene indications and professional categories taking different ward characteristics into account [19]. Two random intercept models were fitted, separately for baseline and follow-up phases, to investigate whether there was significant clustering within wards in relation to hand hygiene compliance, to which extent the variance among wards was explained by ward opportunities mix, and whether specific ward characteristics were associated with compliance variance among wards.

The intra-class correlation (ICC) was used to measure the proportion of the overall variance in hand hygiene compliance explained by the clustering variable. Compliance at baseline in each ward was used to correct the compliance variance estimate. The proportional change in variance (PVC) was used to measure the reduction of variance compared with the empty model or to the previous model. A bivariate analysis was used, overall and also separately for the three types of ward (surgical, intensive care, and medical/other wards) to investigate whether the relative change in hand hygiene compliance was statistically associated with the relative change in ward infrastructure, hand hygiene knowledge and hand hygiene perception questionnaire scores at ward level at follow-up. The relative change in compliance was defined as the absolute difference between two overall measurements (at baseline and follow-up) compared with the baseline measurement in each ward. We applied a linear regression model with bootstrap estimation.

Statistical analyses were performed using STATA/IC 11.1.

Results

Fourteen of 20 regions in Italy agreed to actively participate in the campaign, leading to a total involvement of 175 hospitals comprised of 285 wards. Of the 175 hospitals, 65 returned complete data (37.1%). The variation in participation across regions was not explained by any particular reason. Of the 285 wards, 200 were excluded; 190 (95%) because they were unable to perform all of the requested hand hygiene observations and 10 (5%) because not all the questionnaires were sent back. This left 85 wards (29.8%) remaining for the analysis, which were included. The 65 hospitals included in the analysis were similar to the 110 excluded hospitals in terms of public ownership (83% vs 80%) and infection control score before the campaign (40% vs 39%). The 85 wards included in the analysis were more frequently intensive care units (ICUs) compared with the 172 excluded wards (56% vs 45%, chi-square 3.1, p value = 0.038).

In terms of observed compliance, a total of 18,045 opportunities for hand hygiene were recorded at baseline and 17,577 at follow-up; the number of observation sessions was 1,643 at baseline and 1,403 at follow-up with a median duration of 20 minutes for both baseline and follow-up (IQR: 15 and 10, respectively). The median number of opportunities observed per hour was 20 for baseline and 24 for follow-up (IQR: 24 and 21, respectively). The distribution of observed opportunities was similar during both phases (Table 1).

Of the 65 hospitals included in the 2007–2008 follow-up evaluation, 48 participated in the 2014 HHSAF survey to assess the level of implementation of hand hygiene over time: 40 of 56 public hospitals, six of seven teaching hospitals, and two of two private hospitals.

Impact of campaign, 2007–2008

At baseline, the facility situation median score (measured at baseline only) was high (0.77) and showed small inter-hospital variation (IQR: 0.143), but the ward infrastructure median score was low (0.50) with considerable inter-ward variation (IQR: 0.50). At follow-up, scores arising from the three baseline/follow-up questionnaire areas increased significantly: the ward infrastructure median score increased to from 0.50 to 0.83 (p value < 0.0001), the hand hygiene knowledge median score increased from 0.53 to 0.68 (p value < 0.0001) and the hand hygiene perception median score increased from to 0.69 to 0.77 (p value < 0.0001).

Overall, hand hygiene compliance increased from 40% to 63% (absolute increase: 23%, 95% confidence interval (CI): 22–24%). Compliance significantly increased across all professional categories, types of hand hygiene indications, types of wards and types of hospitals, with the extent of compliance increase being greatest for those areas that showed low compliance before intervention (Table 1).

At baseline, hand hygiene compliance was significantly associated with the type of professional category, the type of hand hygiene indication, the type of ward speciality, and the ward structure for hand hygiene score. Compliance was highest for the professional category of nurses (p < 0.0001), for the indication of 'after body fluid exposure risk' (p value <0.0001), and for medical wards (p value < 0.05) (Table 2). At follow-up, compliance was significantly associated with the type of professional category, the type of hand hygiene indication and the perception score (Table 2).

Notably, a wide variation in hand hygiene compliance among wards was observed at baseline and follow-up. The variance was significant in the empty models both before and after the intervention, and both the before and after ICCs were high (Table 2). At baseline, inter-ward variability of hand hygiene compliance after adjusting for major confounders was seen, with ICUs and medical/other wards having significantly higher compliance than surgical wards (p value = 0.047). This inter-ward variability decreased after campaign implementation, with perception being the only factor significantly associated with this (p value = 0.022). In ICUs and surgical wards, perception was strongly associated with increased hand hygiene compliance at follow-up. Correlating the relative change in overall hand hygiene compliance to the changes in the hand hygiene perception, hand hygiene knowledge and ward infrastructure scores showed that only hand hygiene perception scores were significantly associated with the change (beta-coefficient: 0.343, p value<0.0001).

Long-term sustainability of campaign, 2014

Of the 48 hospitals that completed the HHSAF, the median HHSAF score was 345 (Interquartile range (IQR): 83.7) and 44 were in the intermediate or advanced levels of hand hygiene implementation progress. Overall, the highest component score was for system change (median 100.0, IQR: 20), while the lowest was for institutional safety climate (median 50, IQR: 35). All 12 hospitals that reached the advanced level completed the leadership section; their median leadership score was 14.0 (IQR: 4).

Table 3 describes the 48 hospitals using data collected during the baseline and follow-up evaluations of the 2007–2008 campaign, and data collected during the 2014 HHSAF survey.

Facilities classified as advanced via the HHSAF questionnaire in 2014 were already better performers when the 2007–2008 campaign was initiated: at baseline in 2007–2008, the median scores for hand hygiene compliance (0.52 vs 0.37, chi-squared test 4.28, p value = 0.0384), ward infrastructure (0.67 vs 0.33, chi-squared test 4.17, p value = 0.0411), and hand hygiene perception (0.77 vs 0.69, chi-squared test 7.47, p-value = 0.0063) were significantly higher for facilities classified as advanced compared with those classified as intermediate or basic in 2014. Observed changes between the baseline and follow-up evaluations were comparable between advanced and intermediate/basic facilities with the exception of ward infrastructure for hand hygiene: this improved more among facilities classified as intermediate or basic in 2014 (0.50 median absolute change, IQR: 0.50 vs 0.17 median absolute change, IQR: 0.17, chi-squared test 10.94, p value = 0.0009). In 2014, the median hand hygiene compliance achieved at the end of the 2007–2008 campaign appeared to be sustained. For the 12 hospitals classified as advanced in 2014, the median reported hand hygiene compliance score was 25 points (corresponding to 71–80% compliance) in 2014 while the median observed hand hygiene compliance at follow-up was 74% in 2007–2008. For the 36 intermediate/basic hospitals, the median 2014-reported compliance was 15 points (corresponding to 51–60% compliance) while the median 2007–2008 observed compliance at follow-up was 63% (Table 3).

Discussion

Implementation of a multimodal promotion campaign in 65 hospitals at the national level in Italy led to significant hand hygiene compliance improvement across all types of wards and professional categories.

The inclusion of several hospitals and wards across Italy allowed us to explore factors explaining the variability in hand hygiene compliance among different wards, both before and after campaign implementation. Consistent with previous reports [4], compliance varied across professional categories and types of hand hygiene indications. Nurses started with and achieved the highest level of hand hygiene compliance, consistent with a systematic review of 96 studies that showed median compliance rates 16% lower among physicians compared with nurses [20]. Compliance was highest with the indication 'after contact with body fluids' both at baseline and follow-up. This was consistent with several studies that have demonstrated that hand hygiene action is more frequently performed after contact with body fluids or after patient contact than before, possibly suggestive of self-protection against harmful organisms [4,21].

Compliance increase was indeed accompanied by a parallel improvement in all factors shown to influence hand hygiene behaviour: availability of hand hygiene products within the ward, knowledge of hand hygiene principles, perception of the importance of hand hygiene and of multimodal actions to improve hand hygiene. This is consistent with that reported in other campaigns [9].

The results of the study show that perception characterises the variability in hand hygiene compliance across wards following intervention, and confirms that improving perception of HAIs and hand hygiene improve hand hygiene behaviour.

Others have also found that hand hygiene is influenced by the perceived behaviour of other healthcare professionals [22] and that education methods to enhance perception are requisites for success [18]. In our campaign, several innovative methods were used to promote knowledge and perceptions, such as videos of real-life situations, experiential learning and participatory sessions. Given that others have found that using innovative methods is a key factor for success [21], it is anticipated that the innovative methods used to promote improved knowledge and perception in this study may have been effective at addressing existing behavioural barriers to hand hygiene, especially in settings where compliance was low, such as surgical wards.

Understanding which factors may be important to reducing the variability across wards is of paramount importance to achieving a uniform high level of hand hygiene compliance. Saint et al. found a significant variability of hand hygiene compliance across five wards in Tuscany: the highest performing ward was characterised by the commitment of its physician leaders to hand hygiene improvement and the early adoption of ABHR [23]. Our results confirm both the feasibility and the effectiveness of a large scale implementation of a multimodal hand hygiene promotion campaign, thereby supporting the systematic review and network meta-analysis recently published by Luangasanatip et al [24].

Few studies have reported on long-term hand hygiene compliance [24], especially after a one-off national campaign. The long-term results of the HHSAF survey conducted in 2014 are very encouraging: 7 years after the end of the campaign and without any campaigning activity at the national level thereafter, hospitals that implemented the campaign were still actively promoting hand hygiene. Of the 48 hospitals completing the HHSAF survey, 44 were classified as intermediate or advanced in terms of hand hygiene implementation progress. This compares with 94% (122/129) in the most recent similar survey conducted in the United States using the same tool [13].

All 48 hospitals were still implementing the core components of the WHO campaign even though the campaign itself was not repeated: training scored 40/40 in advanced hospitals vs 20/40 in the other hospitals; hand hygiene direct observation was claimed to still be in place and 71–80% compliance was reported by advanced hospitals vs 51–60% in the others; visible commitment to hand hygiene was assured by top managers scoring 20/20 in advanced hospitals vs 15/20 in the others. Institutions classified as advanced in 2014 were already more prone to hand hygiene promotion at baseline in 2007–2008.

Our study has limitations. First, while we explored the effect of both individual variables, such as professional category and hand hygiene indications, as well as hospital and ward characteristics on compliance, we could not consider additional factors such as the potential positive role of opinion leaders or early adopters. Such investigations are extremely difficult to conduct on a large scale and should be considered as next steps. Second, since hospital participation was voluntary, those participating were possibly more inclined to improve than others. The median facility situation score showing 0.77 at baseline was indeed high with small inter-hospital variability, while Italian national data show significant variation in the development of infection control organisations and initiatives by region and type of hospital [25]. Moreover, we only received full 2007–2008 follow-up data from a subsample of the 175 participating hospitals (37% (n = 65)) mainly due to the unavailability of all the requested data (knowledge, perception, and ward questionnaires and/or hand hygiene observations). Based on the information available, the 65 participating hospitals were similar to the excluded ones in terms of public ownership and infection control activities in place before the campaign. One difference was that the participating hospitals have more ICUs, but the possible effect on the results was minimal. Third, the so called 'Hawthorne effect may have occurred [26]; however, its overall impact is difficult to quantify when the direct observation of practices is conducted and it cannot explain the observed uniform improvement shown across all sites, wards and professional categories in 2007–2008 or the observed sustainability 7 years later. Fourth, this study is limited by the absence of data on patient outcomes, which it was not designed to monitor. Given the high number of enrolled wards not performing HAI surveillance, implementing infection surveillance to evaluate the impact of the campaign on patient outcomes for the duration of the study or using available surveillance data from a proportion of wards only was considered unfeasible and potentially inaccurate. However, a large number of other studies have explored the link between hand hygiene and infection rates [1] and investigators continue to add positive evidence [27-30].

In conclusion, the national campaign using a translated version of the WHO Clean Care is Safer Care materials was effective in improving hand hygiene compliance across 65 hospitals in Italy in 2007–2008: increased perception of HAIs and hand hygiene was an important driver for improvement. The campaign, which was not repeated in following years, seems to have contributed to a good level of hand hygiene 7 years later in 48 of these hospitals.

Acknowledgements

We would like to thank all regional and hospital coordinators, as well as all healthcare workers involved in the national campaign. The 'Clean Care is Safer Care' inter-regional network was composed of: Rosalba Barone (Regione Calabria); Renato Pizzuti (Regione Campania); Maria Luisa Moro, Simona Nascetti, Mita Parenti (Regione Emilia-Romagna); Margherita Vizio, Maura Nannini (Regione Liguria); Marcello D'Errico, Emilia Prospero, Enrica Carducci (Regione Marche); Francesco Bernieri, Anna Pavan (Regione Lombardia);

Carla Zotti, Giacomo Conterno (Regione Piemonte); Peter Josef Santa (Provincia autonoma di Bolzano); Luca Fabbri, Giannina Piccini (Provincia autonoma di Trento); Rosa Prato (Regione Puglia); Paolo Cantaro, Lorenzo Maniaci (Regione Sicilia); Anna Poli, Gaetano Privitera (Regione Toscana); Alessandro Montedori, Maurizio Fiorio (Regione Umbria); Marisa Mastaglia (Regione Valle d'Aosta); Ugo Fedeli, Paolo Spolaore (Regione Veneto). We also want to thank the following hospital coordinators for having contributed to the HHSAF in 2014: Antonioli Paola (Azienda Ospedaliera Universitaria di Ferrara); Argentero Pier Angelo (Azienda Sanitaria Locale TO3 - Distretto di Collegno); Attanasio Giovanni (Azienda Sanitaria Locale TO1 - Distretto Città di Torino); Avanzato Marilena, Bianco Paola (Azienda Sanitaria Locale TO2 - Distretto Città di Torino); Barbaro Silvana, Zozzoli Susanna, Finotto Giuseppe (Azienda Ospedaliera Molinette San Giovanni Battista di Torino); Bedosti Catia (Azienda Usl di Imola); Bellocchi Patrizia (Azienda Ospedaliera Universitaria 'Policlinico Vittorio Emanuele'); Berto Paola (Azienda Sanitaria Locale TO2 - Distretto Città di Torino); Bianchi Emanuela (Azienda Ospedaliera Ospedale di Circolo di Melegnano); Bona Maria Carolina, Macchi Antonella (Azienda Ospedaliera della Provincia di Pavia); Brunelli Davide, Zanella Maria Angela (Ospedale Sacro Cuore Don Calabria - Negrar di Verona); Brusaferro Marco, Marchetto Raffaella (Azienda Ulss 18 di Rovigo); Castellana Marilena, Sisti Marzio, Cesena Rossana, Firetti Anna (Azienda Usl Piacenza); Corrado Vallino, Boux Michela, Musca Rosa (Azienda Sanitaria Locale TO4 - Distretto di Chivasso); Corrado Vallino, Naretto Silvia (Azienda Sanitaria Locale TO4 - Distretto di Ivrea); D'Aloia Francesco (Azienda Sanitaria Locale BI - Biella); Dellalibera Ornella (Azienda Sanitaria Locale AL - Alessandria); Fabbri Luca, Piccini Giannina (Azienda Provinciale Servizi Sanitari di Trento); Farruggia Patrizia (Azienda Usl di Bologna); Fenu Piero, Bagnato Silvia (Azienda Ospedaliera Ordine Mauriziano di Torino); Grossi Paolo (Azienda Ospedaliera Ospedale di Circolo e Fondazione Macchi - Varese); Guareschi Giuliano (Azienda Sanitaria Ospedaliera Regina Margherita - S. Anna - Torino); Lania Donatella (Azienda Ospedaliera Mellino Mellini - Chiari); Marchionni Emilio, Borrini Bianca, Nulletti Tiziana (Azienda Usl Parma); Marinelli Lucia (Azienda Sanitaria Unica Regionale - Zona territoriale 9 di Macerata); Mastaglia Marisa (Azienda USL Valle d'Aosta); Mustara Domenico, Bisello Francesco, Carraro Mara (Azienda Ulss 15 Alta Padovana - Presidio Ospedaliero Camposampiero); Negri Rita (Azienda Ospedaliera Maggiore della Carità di Novara); Ossola Orietta, Pesce Roberta (Azienda Sanitaria Locale VCO - Distretto di Verbania); Parovina Giuseppe, Degiovanni Claudia (Azienda Sanitaria Locale AL Distretto di Casale Monferrato); Pastorelli Emanuela, Scipione Gatti, Franchino Manuela (Azienda Sanitaria Locale VC Distretto di Vercelli); Pellegrino Paolo, Ocelli Paola (Azienda Ospedaliera S. Croce e Carle di Cuneo); Poli Anna (Azienda Usl 10 di Firenze); Pozzetti Cinzia (Azienda Usl di Ravenna); Raglio Annibale (Azienda Ospedaliera Ospedali Riuniti di Bergamo); Rebora Monica, Liggera Luisella (Azienda Sanitaria Locale CN1 Distretto di Savigliano); Rigo Alberto (Azienda Ulss 21 - Legnago); Russo Roberto, Rigobello Vilma (Presidio Sanitario Ospedale Cottolengo di Torino); Sacco Rosa Anna (IRCCS G. Gaslini di Genova); Salvatico Maurizio (Azienda Sanitaria Locale CN1- Mondovì); Tura Grazia Antonella (Azienda Usl di Rimini); Venturino Valentina (Azienda Sanitaria Locale CN2 - Alba).

Financial support provided by Centro per il Controllo e la Prevenzione delle Malattie (CCM), Health Ministry, Rome, Italy.

Conflict of interest

None declared.

Authors' contributions

B Allegranzi, S Nascetti, MG Pompa, D Pittet and ML Moro contributed to the design of study. S Nascetti, M Parenti and F Morsillo provided the collection and assembly of data. F Morsillo performed the data analysis. ML Moro and F Morsillo wrote the manuscript. B Allegranzi, D Pittet and ML Moro contributed with critical revision and reviewed the manuscript.

References

1. Allegranzi B, Pittet D. Role of hand hygiene in healthcare-associated infection prevention.J Hosp Infect. 2009;73(4):305-15. DOI: 10.1016/j.jhin.2009.04.019 PMID: 19720430
2. World Health Organization (WHO). Evidence of hand hygiene to reduce transmission and infections by multi-drug resistant organisms in health-care settings. Geneva: WHO. [Accessed 21 May 2017]. Available from: http://www.who.int/gpsc/5may/MDRO_literature-review.pdf
3. Pittet D, Donaldson L. Clean Care is Safer Care: the first global challenge of the WHO World Alliance for Patient Safety. Infect Control Hosp Epidemiol. 2005;26(11):891-4. DOI: 10.1086/502513 PMID: 16320985
4. World Health Organization (WHO). WHO Guidelines on Hand Hygiene in Health Care: a Summary. First Global Patient Safety Challenge. Clean Care is Safer Care. Geneva: WHO; 2009.Available from: http://apps.who.int/iris/bitstream/10665/70126/1/WHO_IER_PSP_2009.07_eng.pdf
5. World Health Organization (WHO). Guide to Implementation: A Guide to the Implementation of the WHO Multimodal Hand Hygiene Improvement Strategy. Geneva: WHO; 2007, revised in 2009. Available from: http://www.who.int/infection-prevention/publications/hh_implementation-guide/en/
6. World Health Organization (WHO). Clean Care is Safer Care: European countries statements. Geneva: WHO. [Accessed 30 May 2017]. Available from: http://www.who.int/gpsc/statements/countries/EN_PSP_GSPC1-EURO_Country/en/
7. World Health Organization (WHO). Clean Care is Safer Care: Tools and Resources. Geneva: WHO. [Accessed 10 February 2015]. Available at: http://www.who.int/gpsc/5may/tools/en
8. World Health Organization (WHO). Campagna 'Cure pulite sono cure più sicure'. ['Clean Care is Safer Care' Campaign]. [Accessed 22 March 2017]. Italian. Available from: http://assr.regione.emilia-romagna.it/it/ricerca-innovazione/prevenzione-antibioticoresistenza-infezioni/sorveglianza-controllo/controllo-rischio-infettivo/igiene-mani/cure-pulite-strumenti/intro
9. Allegranzi B, Gayet-Ageron A, Damani N, Bengaly L, McLaws ML, Moro ML, et al. Global implementation of WHO's multimodal strategy for improvement of hand hygiene: a quasi-experimental study. Lancet Infect Dis. 2013;13(10):843-51. DOI: 10.1016/S1473-3099(13)70163-4 PMID: 23972825
10. Sax H, Allegranzi B, Uçkay I, Larson E, Boyce J, Pittet D. 'My five moments for hand hygiene': a user-centred design approach to understand, train, monitor and report hand hygiene.J Hosp Infect. 2007;67(1):9-21. DOI: 10.1016/j.jhin.2007.06.004 PMID: 17719685
11. Sax H, Allegranzi B, Chraïti MN, Boyce J, Larson E, Pittet D. The World Health Organization hand hygiene observation method. Am J Infect Control. 2009;37(10):827-34. DOI: 10.1016/j.ajic.2009.07.003 PMID: 20004812
12. World Health Organization (WHO). Hand Hygiene Self-Assessment Framework 2010. Geneva: WHO. [Accessed 22 March 2017]. Available from:http://www.who.int/gpsc/country_work/hhsa_framework_October_2010.pdf?ua=1
13. Allegranzi B, Conway L, Larson E, Pittet D. Status of the implementation of the World Health Organization multimodal hand hygiene strategy in United States of America health care facilities.Am J Infect Control. 2014;42(3):224-30. DOI: 10.1016/j.ajic.2013.11.015 PMID: 24581011
14. Goldstein H. Multilevel Statistical Models. 2nd ed. London: Edward Arnold, 1995: 116-120.
15. Hox J. Multilevel Analysis: Techniques and Applications. 2nd ed. East Sussex: Routledge, 2010: 103-122.
16. Rabe-Hesketh S, Skrondal A. Multilevel and Longitudinal Modeling Using Stata. 2nd ed. Texas: Stata Press, 2008: 247-252; 444-453.
17. Pittet D, Hugonnet S, Harbarth S, Mourouga P, Sauvan V, Touveneau S, et al. Effectiveness of a hospital-wide programme to improve compliance with hand hygiene. Lancet.

2000;356(9238):1307-12. DOI: 10.1016/S0140-6736(00)02814-2 PMID: 11073019

18. Pittet D, Simon A, Hugonnet S, Pessoa-Silva CL, Sauvan V, Perneger TV. Hand hygiene among physicians: performance, beliefs, and perceptions.Ann Intern Med. 2004;141(1):1-8. DOI: 10.7326/0003-4819-141-1-200407060-00008 PMID: 15238364
19. Littell RC, Milliken GA, Stroup WW, Wolfinger RD. SAS System for Mixed Models. Cary, North Carolina: SAS Institute Inc., 1996: 253-266
20. Erasmus V, Daha TJ, Brug H, Richardus JH, Behrendt MD, Vos MC, et al. Systematic review of studies on compliance with hand hygiene guidelines in hospital care. Infect Control Hosp Epidemiol. 2010;31(3):283-94. DOI: 10.1086/650451 PMID: 20088678
21. Mathai E, Allegranzi B, Seto WH, Chraïti MN, Sax H, Larson E, et al. Educating healthcare workers to optimal hand hygiene practices: addressing the need. Infection. 2010;38(5):349-56. DOI: 10.1007/s15010-010-0047-7 PMID: 20857314
22. Erasmus V, Brouwer W, van Beeck EF, Oenema A, Daha TJ, Richardus JH, et al. A qualitative exploration of reasons for poor hand hygiene among hospital workers: lack of positive role models and of convincing evidence that hand hygiene prevents cross-infection. Infect Control Hosp Epidemiol. 2009;30(5):415-9. DOI: 10.1086/596773 PMID: 19344264
23. Saint S, Bartoloni A, Virgili G, Mannelli F, Fumagalli S, di Martino P, et al. Marked variability in adherence to hand hygiene: a 5-unit observational study in Tuscany. Am J Infect Control. 2009;37(4):306-10. DOI: 10.1016/j.ajic.2008.08.004 PMID: 19135761
24. Luangasanatip N, Hongsuwan M, Limmathurotsakul D, Lubell Y, Lee AS, Harbarth S, et al. Comparative efficacy of interventions to promote hand hygiene in hospital: systematic review and network meta-analysis. BMJ. 2015;351:h3728. DOI: 10.1136/bmj.h3728 PMID: 26220070
25. Moro ML, Marchi M, Buttazzi R, Nascetti S, INF-OSS Project Group. Progress in infection prevention and control in Italy: a nationwide survey.J Hosp Infect. 2011;77(1):52-7. DOI: 10.1016/j.jhin.2010.08.009 PMID: 21131101
26. Whitby M, McLaws ML. Methodological difficulties in hand hygiene research.J Hosp Infect. 2007;67(2):194-5. DOI: 10.1016/j.jhin.2007.08.002 PMID: 17884247
27. Barnett AG, Page K, Campbell M, Brain D, Martin E, Rashleigh-Rolls R, et al. Changes in healthcare-associated Staphylococcus aureus bloodstream infections after the introduction of a national hand hygiene initiative. Infect Control Hosp Epidemiol. 2014;35(8):1029-36. DOI: 10.1086/677160 PMID: 25026620
28. Stone SP, Fuller C, Savage J, Cookson B, Hayward A, Cooper B, et al. Evaluation of the national Cleanyourhands campaign to reduce Staphylococcus aureus bacteraemia and Clostridium difficile infection in hospitals in England and Wales by improved hand hygiene: four year, prospective, ecological, interrupted time series study. BMJ. 2012;344(3 May 2012):e3005.PMID: 22556101
29. Ho ML, Seto WH, Wong LC, Wong TY. Effectiveness of multifaceted hand hygiene interventions in long-term care facilities in Hong Kong: a cluster-randomized controlled trial.Infect Control Hosp Epidemiol. 2012;33(8):761-7. DOI: 10.1086/666740 PMID: 22759542
30. Allegranzi B, Harbarth S, Pittet D. Effect of Hand Hygiene on Infection Rates. In: Pittet D, Boyce JM, Allegranzi B, editors. Hand Hygiene: A Handbook for Medical Professionals. Chichester: John Wiley and Sons, 2017.

License and copyright