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Letters to the Editor

Persistent norovirus outbreaks in a hospital setting – The role of environmental contamination

Dear Editor,

A recent review highlighted the healthcare burden of norovirus infections in China, prior to 2017. Norovirus infections occurred all year round, with 22–29% detection rates in children (6–35 months old) and adults (including the elderly), predominantly of norovirus GII.4 (70.4%).[1] Similarly, in England and Wales in 2016 there were 7795 laboratory reported cases of norovirus and acute gastroenteritis, which was estimated to cost the National Health Service up to £86 million annually in bed closures and staff absences,[2,3] making norovirus the second-largest contributor of gastrointestinal disease hospital burden in England.[4]

Norovirus is the world's leading cause of gastroenteritis. Three of its seven genogroups (GI, GII, GIV) cause human infections and GII.4/Sydney/2012 is the current, dominant lineage globally. Transmission is faeco-oral, resulting in nosocomial outbreaks of diarrhoea and vomiting, typically peaking during winter. Multiple norovirus cases and outbreaks have occurred at our hospital throughout the non-winter months over several years, suggesting possible environmental contamination and reinfection of patients.[5] To investigate this, a retrospective analysis of recent norovirus cases and selected environmental sampling on an outbreak ward were performed.

All laboratory-confirmed (on real-time PCR testing) norovirus positive clinical and environmental samples (with sufficient sample volume) from Jan2017-Jun2018 were included, and sent to the national reference laboratory (Enteric Virus Unit, PHE) for genotyping by sequencing methods used for national surveillance.[6] Partial norovirus capsid protein VP1 (ORF2) sequences from these samples were aligned and edited with similar GenBank sequences. Maximum likelihood phylogenetic trees were constructed to reveal any correlation between the patient and environmental samples.

A total of 216 samples (mostly from general medical wards) were laboratory-confirmed norovirus positives during 2017–2018, of which 133 (41/66 from 2017; 92/146 from 2018) were sequenced. Of these, 6/216 (2.78%) were GII.7 and 127/216 (58.80%) were GII.4 viruses. The remainder of the samples (83/216, 38.42%) could not be sequenced, either due to insufficient sample remaining, the sample having already been discarded, or a sequencing failure at the reference laboratory. Sequences consisted of partial VP1 sequences of approximately 300 bp in length.

In addition, environmental swabs (see Fig 1) were taken for norovirus detection and sequencing from one of the worst affected wards (Ward X, a care of the elderly ward). This included pre- and post-clean samples (Fig. 1a,b) taken from one shared 6-bedded bay, one shared patient bathroom and two single patient isolation rooms. Routine cleaning for norovirus was performed manually on all surfaces, using clean, single-use, cloths dipped in a commercial troclosene sodium (sodium dichloroisocyanurate, NaDCC)-based cleansing agent ('Chlor-Clean', Guest Medical Ltd., Kent, England) at a concentration of 1000 parts per million. Both patient and environmental samples were taken using a commercial swab made of polyurethane foam collected into proprietary virus transport medium (Sigma Virocult, MWE Ltd., Corsham, England).

Of the total number of environmental swabs taken (100: 47 pre- and 53 post-clean), 28/100 were positive for norovirus RNA and contained sufficient virus for sequencing, of which 21/28 (75%) were pre-clean (all taken on 7/3/2018), and 7/28 (25%) were post-clean (all taken on 22/3/2018). None of the Ward X patients that were on this ward during this study period were actually admitted with vomiting and diarrhoea. Therefore these norovirus infections were acquired from the ward, either sporadically or during a ward outbreak, after their admission for other reasons.

The top of the phylogenetic tree (**Fig. S1**, purple font) shows that separate GII.7 virus outbreaks occurred on the adult haematology (Ward H, Sep2017) and paediatric respiratory (Ward P2, Feb/Mar2018) wards, not thought to be epidemiologically linked. The UHL sequences are distinct (SH support 0.75) from the closest similar sequence from GenBank (black). No environmental samples were taken from these wards. However, the majority of the UHL GII.4 patient (blue) and Ward X environmental viruses detected during Dec2017-May2018 are most closely related to a 2016 GII.4 virus from Thailand, detected in human stool. Only 15 pre-clean (red) and 5 post-clean (green) environmental, and 86 patient samples were of sufficient sequence quality for inclusion in this final phylogenetic tree. Note that all virus detection was performed by PCR testing, which does not give any indication of virus viability.

One subset of UHL GII.4 patient viruses detected during Dec2017-Feb2018 are most closely related to a 2016 GII.4 virus from Bangladesh (detected in sewage) or a 2015 GII.4 virus from Australia, detected in human stool. A second subset of UHL GII.4 patient viruses detected during Jul2017 and Feb2018 appear to be more closely related to 2015/2016 GII.P16 GII.4 viruses from the USA, detected in human stool.

A closer examination of **Fig. S1** (i.e. within the red boxes) shows that very similar viruses were found across several hospital wards during Mar2017-May2018, suggesting the long-term persistence of these viruses during this 15-month period, in either the environment and/or hospitalised patient population. Such long-term viral persistence may potentially infect future patients being admitted to these wards and this hospital.[7] Deeper analysis of genetic similarity was limited by the use of an existing partial sequencing approach that was designed for another purpose (national

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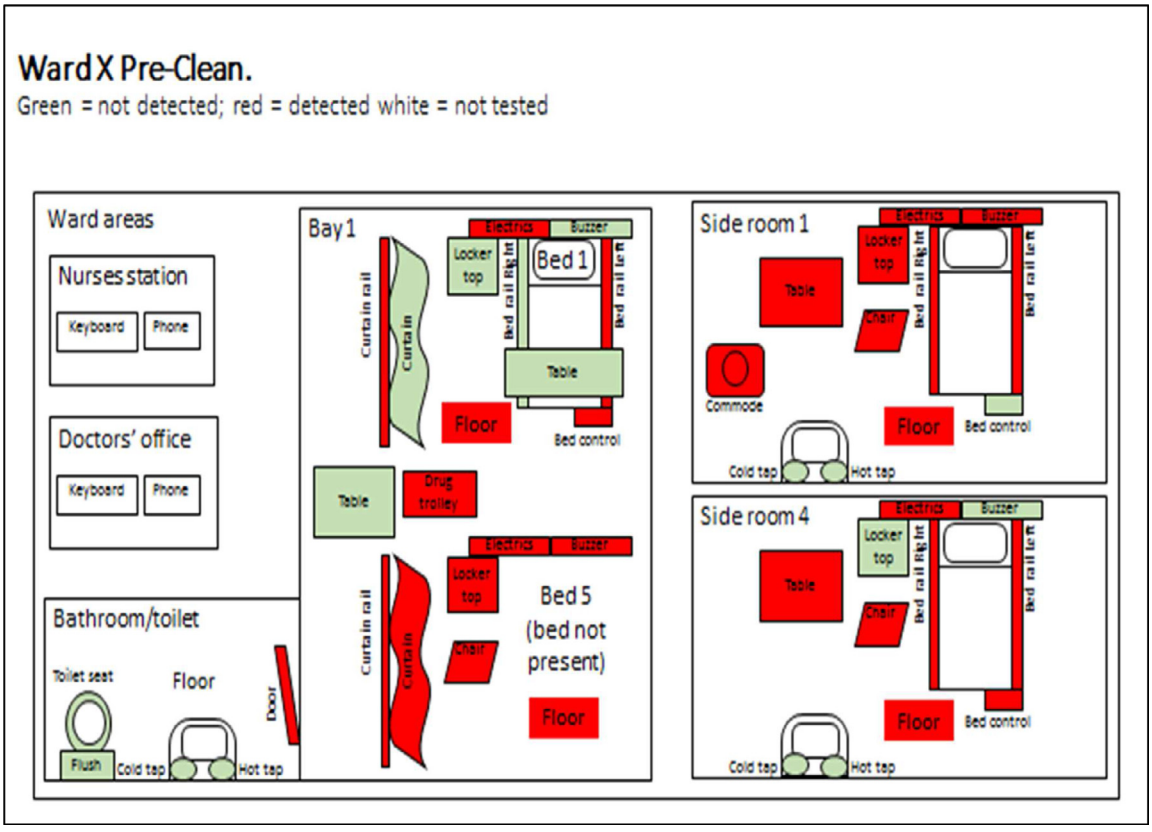

Fig. 1a. Ward map showing layout and contents of patient rooms with selected sites for environmental sampling – before cleaning.

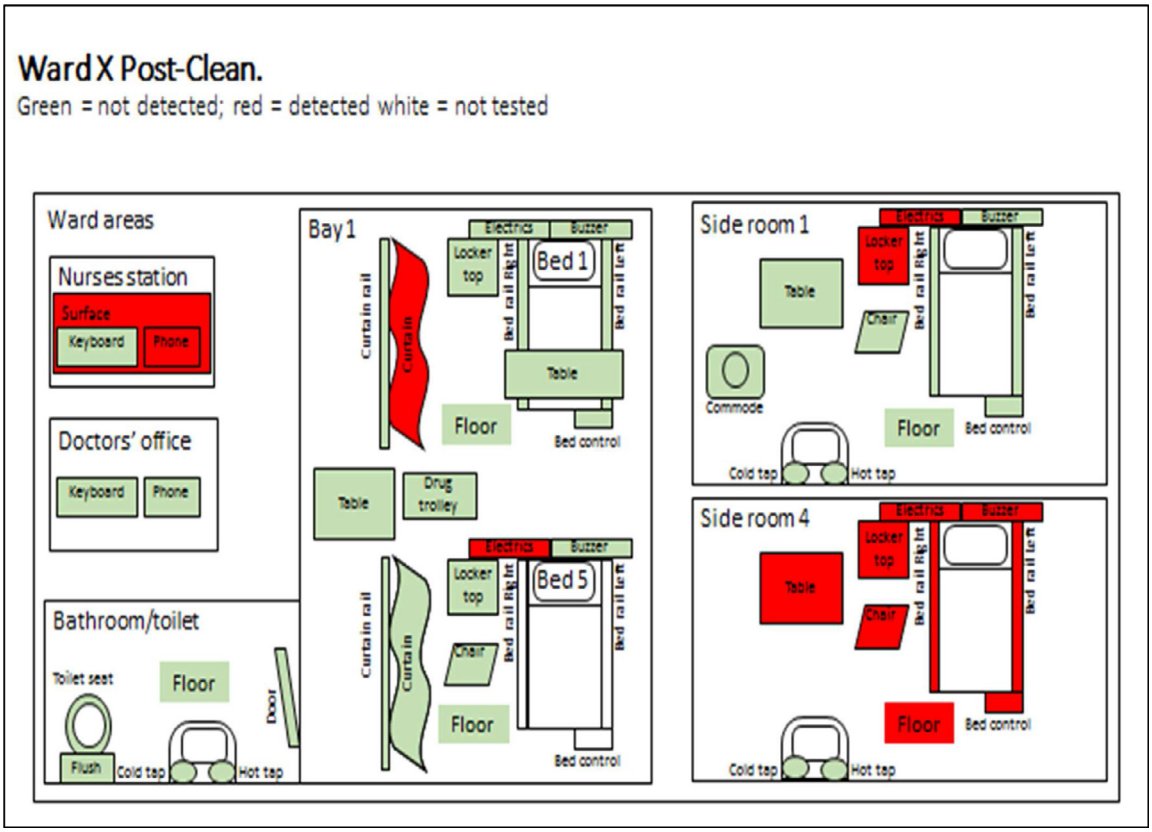

Fig. 1b. Ward map showing layout and contents of same patient rooms with selected sites for environmental sampling – after cleaning.

surveillance); partial sequencing of both polymerase and capsid genes, or whole genome sequencing, could provide greater resolution in investigating potential transmission chains in the future.[8–10]

Therefore, the main findings from this investigation are (i): both GII.4 and GII.7 noroviruses have been detected in UHL patients during Jan2017-Jun2018; (ii) some of the patients from the 2017 and 2018 outbreaks were infected with very similar viruses, suggesting persistence of these same viruses in the environment or patient population for over a year; (iii) the ward environmental contamination with norovirus may persist after deep cleaning.

As a result of the last finding, since mid-2018, we have reviewed and enhanced our ward cleaning methods at our hospital. One component of this included updating our hospital 'norovirus toolkit' which includes team action cards that identify the type of clean required. These now also include items that might have been missed previously, e.g. replacing patient anti-slip socks and the cleaning of any hospital-related patient footwear (e.g. Repose Boots) when they are moved into a clean bed-space.

Conflict of interest

None.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2019.06.002.

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Is the analysis sufficient? Letter to the Editor concerning the study of Prof. Wang titled "Xpert MTB/RIF Ultra improved the diagnosis of paucibacillary tuberculosis: A prospective cohort study" ☆

Dear Editor,

We read with great interest the recent study of Prof. Wang regarding the Xpert MTB/RIF Ultra improved the diagnosis of paucibacillary tuberculosis.[1] Due to the limitations of the use of Xpert in patients with sputum smear-negative or extrapulmonary tuberculosis or people co-infected with HIV, WHO has recommend using the Xpert Ultra for TB diagnosis and suggested to extensively evaluate Xpert Ultra in different epidemiological and geographical settings with different patient populations since March 2017.[2] This study[1] demonstrated that Xpert Ultra outperformed Xpert by comparing the diagnostic performance on a large number of paucibacillary sputum and non-respiratory specimens in China, such a TB high-burden and HIV low-burden setting. We appreciate this frontier exploration. However, we are puzzled about some small issues on the data presentation, conduction and analysis, which may affect the accuracy and completeness of a diagnostic report.

First, when sputum and pleural fluid were analysed for the Yield of Xpert Ultra in rifampicin resistance detection, the authors stated[1] that overall sensitivity of Xpert Ultra (100%, 22/22) was slightly higher than Xpert (95.45%, 21/22, $P = 0.312$) in pleural fluid samples, though the difference was not significant. Based on the other part of this article, we thought this may be an writing error, cause this sensitivity value was calculated as a result of all specimens included in this detection.

☆ There's no Conflict of Interest disclosures.

Second, they reclassified all the trace-positive as tuberculosis-negative outcome for sputum examination to avoid evident decrease of specificity.[1] However, Dorman et al.[3] observed a decreased specificity for Xpert Ultra in patients with a medical history of TB treatment compared to those without, which could be affected by the time since last end of TB treatment. So the reclassification of trace-positive as tuberculosis-negative in Wang's results may be hasty, as patients with history of tuberculosis were 49.6% of all PTB,[1] which could be conducted through a subgroup analysis for patients with a tuberculosis treatment history only (a conditional-trace approach), or considered on the basis of Xpert Ultra test results from another sputum specimen (a trace-repeat approach). The two approaches retained most of Xpert Ultra's sensitivity in the smear-negative group.

Third, the diagnostic performance of Xpert Ultra on any kind specimens used the composite reference standard (CRS) as control including the Xpert Ultra results, which could cause incorporation bias.[4] The absence of a true gold standard for tuberculous meningitis diagnosis or absence of post-mortem examinations was also a potential concern.[4]

Forth, not the whole sample or a random selection of sample received the microbiological detections including smear microscopy, culture and Xpert or Xpert Ultra as part of CRS contents and it is unclear on sample numbers and definitions of histopathological/thoracoscopic or radiological examinations. This could result in partial verification biaswork-up bias.[5]

Fifth, the distribution of diseases severity was not reported, lacking of the diagnostic performance between different subgroups, which may bring about disease progression bias.[5]

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Some doubts on the meta-analysis of the clinical significance of thrombocytopenia complicating sepsis

Dear Editor,

We read with great interest the recently published meta-analysis by Xie et al.,[1] who concluded that thrombocytopaenia was associated with poor prognosis in patients with sepsis. Though their study sounds scientific, however, we wish to raise some questions about the study.

First, in the second paragraph of the meta-analysis, Xie et al. claimed that 16 articles were finally included - 12 articles were English-language literature and 4 articles were Chinese-language literature. However, according to Table 1, it seems that 17 rather than 16 studies had been incorporated and the number of English-language literature included should be 13 rather than 12.[1]

Second, Dr. Xie simply used the term "sepsis" to identify eligible studies, nevertheless, this search term is too simple and not comprehensive and some important studies could have been undetected. In fact, severe sepsis and septic shock are serious types of sepsis and should be taken into consideration.[2,3]

Third, Xie et al. aimed to investigate the population with sepsis, however the study leaded by Vandijck in 2010 was a retrospective cohort study of patients with bloodstream infection. As we all know, sepsis is a combination of infection and systemic inflammatory response syndrome,[2,3] which meant that not all patients in the study carried by Vandijck were septic, thus this cohort study should not be included.

Fourth, after the comparison of the sequence number of the included studies in Table 1 with the sequence number of the reference, we found that they two did not match each other, therefore the authors should check their reference and modify it carefully.

Fifth, when the authors of the meta-analysis found the heterogeneity between studies was large, they chose the random-effects model, this was proper, but the source of heterogeneity should be investigated, perhaps subgroup analysis on the base of the characters of the included studies (such as country, sample size, number of centre, study type or ICU type) together with meta-regression would help.[4] However, when the heterogeneity between studies was not heterogeneous ($I^2 = 0\%$), they still chose the random-effects model, it did not seem proper, fixed-effects model rather than random-effects model would be more accurate.

Finally, we appreciate Xie et al. for their innovative work, but further rigorous studies are still required.

Conflict of interest declaration

The authors declare that there are no conflicts of interest.

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Abbreviations: ICU, intensive care unit.

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Genomic epidemiology of *Neisseria meningitidis* serogroup W in Switzerland between 2010 and 2016

Dear Editor,

We read with great interest the paper on 'Clonal replacement and expansion among invasive meningococcal isolates of serogroup W in France' by Hong, E; Barret, AS et al. published in this journal.[1]

The incidence of *Neisseria meningitidis* of serogroup W (MenW) is increasing in Europe.[2] There is a particular interest on MenW clonal complex (cc) 11, a hyperinvasive lineage, because it caused several outbreaks worldwide during the last decade.[3,4] Two main W cc11 sub-lineages have been characterized so far and are considered of interest from a public health perspective because of their virulence.[3] One lineage contains isolates of a South American strain which expanded from Brazil to the UK and Europe.[5] This lineage emerged in the UK in 2009 and evolved over 2009–2013 in the UK (novel UK 2013). The other lineage includes strains related to the Saudi-Arabian outbreak that occurred in 2000 during Hajj pilgrimage.[4]

In Switzerland, 38–73 invasive meningococcal disease cases occur each year (corresponding to an annual incidence rate of 0.46–0.92 cases per 100,000 inhabitants[6]). Since 2011, the number of reported MenW cases has increased steadily over time (Fig. 1(A)): MenW caused 2% of cases in 2011 but 35% in 2015[6] and in 2016 serogroup W became the second most frequently identified invasive meningococcus (Fig. 1(A)[7]). In total 40 MenW were sent to the Swiss National Reference Center for Meningococci during the 2010–2016 period. Like for France, we observed that in Switzerland clinical manifestations of MenW cases included mostly sepsis (58%) and meningitis (23%), but rarely arthritis (10%). Moreover, in 2015–2016 MenW was especially prevalent among individuals older than 15 years (64.3% and 91.7% of cases in 2015 and in 2016, respectively).

Since 2013, Sequence Type (ST) 11, which belongs to cc11, was the most frequent in years 2014 and 2016, and in 2016 the only ST detected (Fig. 1(B)). Other sequence types, ST-184, ST-22, ST-1221 and ST-1286, all belonged to cc22 and remained sporadic over the same period.

Like French colleagues, we applied whole genome shotgun sequencing (WGS) to investigate clonal relationships among those 40 Swiss MenW isolates and 78 European reference genomes collected from previous studies[3,8,9] and that are representative of the two cc11 sub-lineages mentioned above.

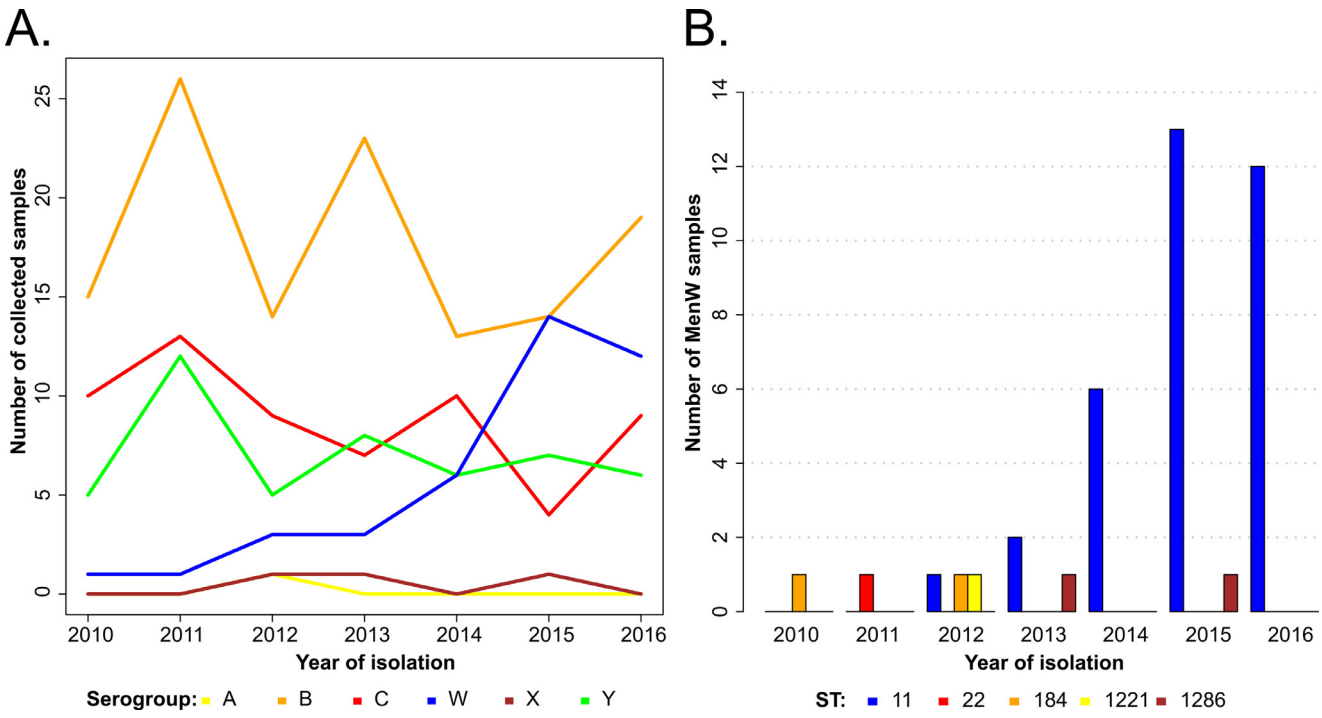

Fig. 1. (A) Distribution of samples by serogroup according to the year of isolation. Colors are according to the serogroup type. (B) Distribution of the 40 MenW Sequence Types (STs) according to year of isolation. Colors are according to the ST.

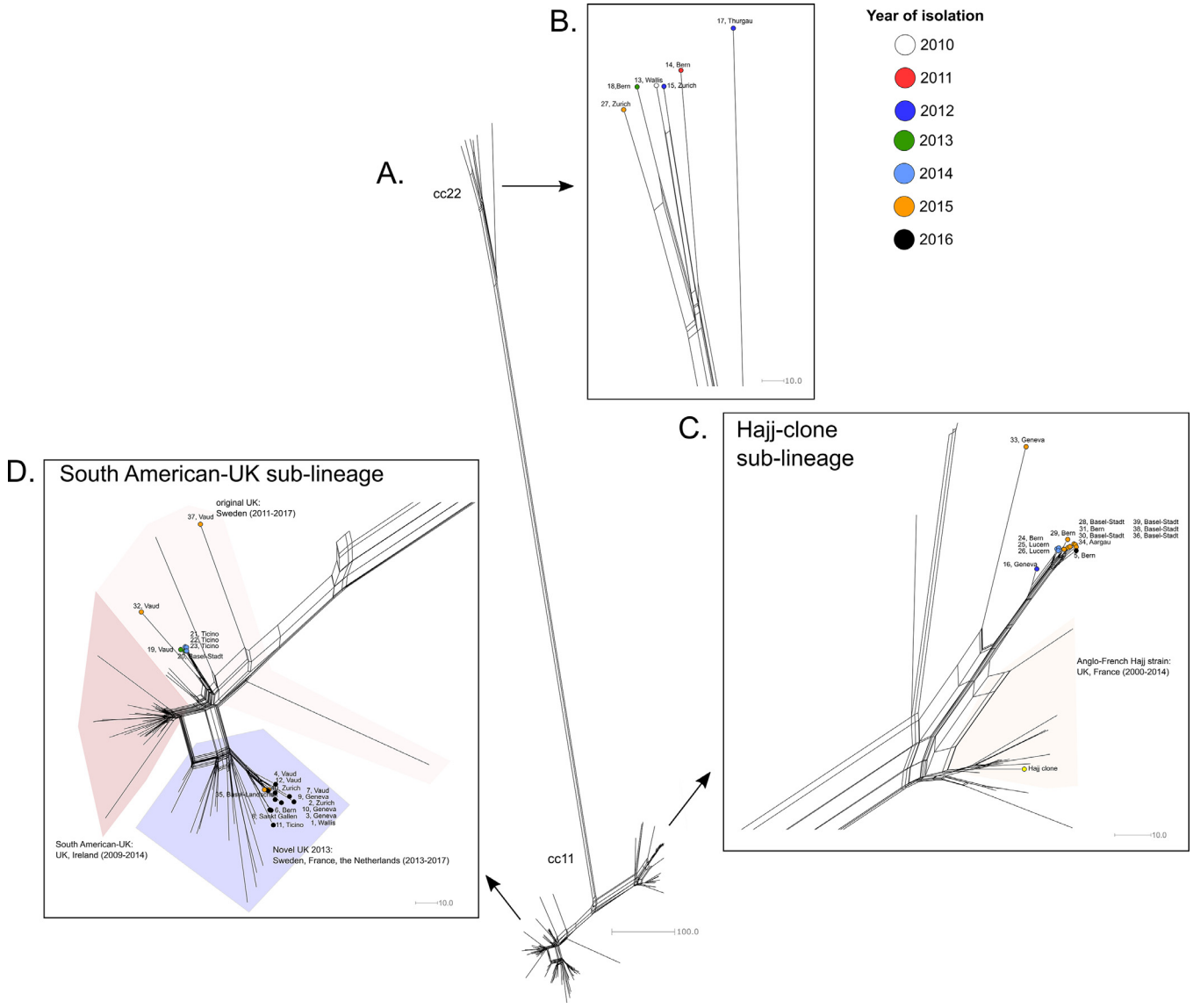

Fig. 2. Neighbor-net phylogenetic networks based on the comparison of 1605 core genome loci from PubMLST *Neisseria* database (https://pubmlst.org/neisseria/). Analyses were performed in BIGSdb Genome Comparator tool implemented within the PubMLST website with default settings. Incomplete loci, due to an incomplete genome assembly, were not considered in pairwise comparisons. B, C and D. represent magnified sections of the network showed in A. In B.: cc2 Swiss MenW samples are reported. In C.: Swiss and European samples related to the Hajj-clone (marked in yellow) are shown. Colored shadow represent samples from UK and France of Anglo-French Hajj strain. Other reference genomes come from Sweden and are reported to belong to Hajj-clone lineage (see below). In D.: Swiss and European genomes from South-American-UK original lineage (grouped in the red shaded polygons) and from the novel UK 2013 sub-lineage (grouped in the blue shaded polygon) are reported. Swiss samples are labelled according to Sequence Identifier followed by canton of isolation and they are colored according to the year of isolation. Scale bars indicate the number of allelic differences. Further information concerning the reference strains are available through their PubMLST IDs (Anglo-French Hajj strains from[3]: 31164, 31165, 31167, 29696, 29697, 29699, 29700, 29702, 29703, 29966, 29967, 30266, 30267; Swedish samples from Hajj sub-lineage from[8]: 42425, 47180, 47181, 47182, 47186, 50975, 52796, 52797, 53776; Hajj clone from[4]: 2290; South American-UK strain from[3]: 26898, 26899, 26914, 19968, 20154, 20158, 20196, 20216, 20226, 20247, 20288, 20368, 20436, 20444, 20449, 21123, 21163, 21203, 21206, 21214, 28128, 28131, 29714,29715, 29716, 29718, 29719, 29720, 29813, 29814, 30152, 30153, 30167, 30170; Original UK lineage from[8]: 39601, 41966, 47206, 47208, 50872; Novel UK 2013 lineage from[8,9]: 52131, 53526, 38691, 44573, 50353, 38602, 38614, 39602, 39603, 41963, 41964, 42363, 42391, 42420, 51910, 53033).

We observed that samples clustered according to clonal complexes and that cc22 isolates showed higher diversity than cc11 samples (Fig. 2). cc11 isolates were distributed in two main branches corresponding to Hajj-clone and South-American related strains (Fig. 2(C) and (D)).

The Hajj-clone related branch included 14 Swiss MenW collected between 2012 and 2016. However, the majority of these samples were isolated in 2014 (3/14) and 2015 (9/14). Excluding sample 33, which was found as the most different from the other samples, all others Swiss isolates distributed along Neighbor-net network in a chronological order (from left to right, Fig. 2(C)) likely reflecting an evolution history from the Hajj-clone (year 2000), via strain 16 (year 2012), towards other members of this cluster (years 2014–2016) (Fig. 2(C)).

We found that the remaining 20 cc1 Swiss isolates distributed within the South American sub-lineage (Fig. 2(D)). 7 out of 20 isolates were more related to Swedish original-UK samples and other South-American-UK samples than the other 13 cc1 were (Fig. 2(D)). Of note 3 out of 7 were collected in the canton of Ticino in 2014.

The other 13 samples were related to the novel UK 2013 lineage. 11 out of 13 samples were collected in 2016 mostly from French-speaking neighbouring cantons Geneva and Vaud (Fig. 2(D)). Those isolates were closely-related to the two samples isolated in 2015 in the German-speaking cantons of Basel-

Landschaft and Zürich. Therefore, the 11 2016-samples likely resulted from the expansion of a clone already circulating in Switzerland a year before.

In conclusion, we provide evidence that the cc11 Hajj-clone- and related South-American-UK sub-lineages have been circulating in Switzerland as shown in France and other European countries (24, 25). Our results support those provided by Hong, E; Barret, AS et al. showing that increase of the MenW cases is characterized by a replacement of Hajj-clone-related strains with those of South-American sub-lineages which occurred in Switzerland between 2015 and 2016. Of note, like in France, we found a strong expansion of novel-UK-2013 strain in 2016.

Importantly, the incidence of invasive MenW in Switzerland is still increasing: it was the most frequent *N. meningitidis* serogroup detected in 2017[10] and 2018 (Swiss National Reference Center for Meningococci – 2018 Annual Report; in preparation). Therefore, the genomic analyses of 2017–2018 MenW samples would allow to monitor and to follow up the genetic evolution of lineages circulating in the country.

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Revealing true diversity of measles viruses circulating in India, 2012–17

Dear Editor,

In this journal, Ahmed and colleagues drew attention to the resurgence of measles and the specter of vaccine failure.[1] In India, measles virus (MeV) genotyping is based on the carboxyl-terminal of nucleoprotein (N) and or hemagglutinin (H) gene and subsequently classified into A-H clades and 24 sub-clades or genotypes.[2] Recent reports had revealed that the H and matrix-fusion (M/F) non-coding region had greater variability than N gene.[3,4] The genetic characterization of MeV is essential to measure the impact of immunization with context of measles elimination strategies in India.[5,6] Largely, MeV D4 and D8 strains are circulating in different parts of India.[7] Therefore, a study was formulated to determine diversity of MeV genotypes using M/F region. Sixty-seven MeV isolates obtained during year 2012–17 from 11 states were included in the study. These isolates (in Vero hSLAM cells) were obtained from the patients presented with 1–3 days fever with maculopapular rashes.

Viral RNA was extracted from each virus aliquot using QIAmp viral RNA minikit (Qiagen). For amplifying the MeV M/F region, three pairs of primers were used (Available on request). Five micro liter of extracted RNA was used to amplify the M/F region using Super-Script III One-Step RT-PCR with Platinum Taq DNA polymerase (Invitrogen). For the N and H gene sequencing, previously reported primers and conditions were used.[8,9] The PCR products were purified by ExoSAP-IT reagent (Affymetrix). Two micro liter of DNA template added into 18 µL sequencing reaction prepared using BigDye™ terminator v3.1 kit (Applied Biosystems) and respective primer set. Sequencing reaction was purified using the DyeEx 2.0 spin kit (Qiagen) and sequences obtained by automated DNA analyzer (ABI 3730XL, DNA analyzer). A consensus sequence of each gene was taken for analysis and subsequently deposited in GenBank (MG664549-569 and MG721157-205).

The nucleotide sequences (i.e. N, H and M/F) of different MeV genotypes were included in the phylogenetic analysis. MEGA v6 was used for the multiple sequence alignment, determination of best DNA model and reconstruction of Maximum Likelihood (ML) phylogenetic tree.[10] The best nucleotide substitution model selected based on AICc value. The ML tree was inferred by using heuristic search method with extensive level of Subtree-Pruning-Regrafting algorithm. The mean percent nucleotide divergences (PND) within/between different genotypes were calculated. BEAST v1.8.2[11] was used for calculating the mean substitution rates. Re-

laxed clock model with lognormal distribution and constant coalescent growth model were used and convergence was assessed using Tracer v.[11]

For the 67 MeV isolates, three genes were sequenced that resulted in a final consensus fragments of 450, 1854 and 1012 nucleotides for N, H and M/F genes, respectively. Phylogenetic analysis with the WHO reference N, H and M/F sequences revealed similar genotype grouping i.e. 11 D4 and 56 D8 genotypes. Different crucial sites (i.e. various epitopes and binding sites) were mapped on H gene. Thirteen Indian MeV isolates showed I473V mutation in sugar-shielded epitope, twenty isolates showed S247P mutation in the neutralizing epitope, four isolates showed S313A or S313L mutations at loop epitope and only one MeV isolate showed N396D mutation at haemaglutinating and neutralizing epitope.

For M/F gene, GTR+G was the best model while GTR+G+I was that for N gene. TN93+G was the best model for H-gene dataset. The mean rate of nucleotide substitution for M/F gene was found to be 1.03×10^{-2} (95% HPD: 4.3×10^{-3}, 1.65×10^{-2}); N-gene was 9.62×10^{-4} (95% HPD: 4.77×10^{-4}, 1.44×10^{-3}) and for H-gene was 7.58×10^{-4} (95% HPD: 4.57×10^{-4}, 1.10×10^{-3}). The topology of ML trees for both the genes was similar with high bootstrap supports (>70%) for all nodes representing monophyletic groups forming different genotypes. The ML tree for M/F gene (Fig. 1) demonstrated clear differentiation between different genotypes like that for N gene and H gene (Supplementary Figs. 1 and 2). The overall mean percent divergence was slightly higher for M/F gene (7.7%) compared to N gene (3.9%) and H gene (1.9%) indicating added variability in M/F gene. For M/F gene, the within genotypes mean PND ranged from 2.5 to 4.8% while the between genotypes

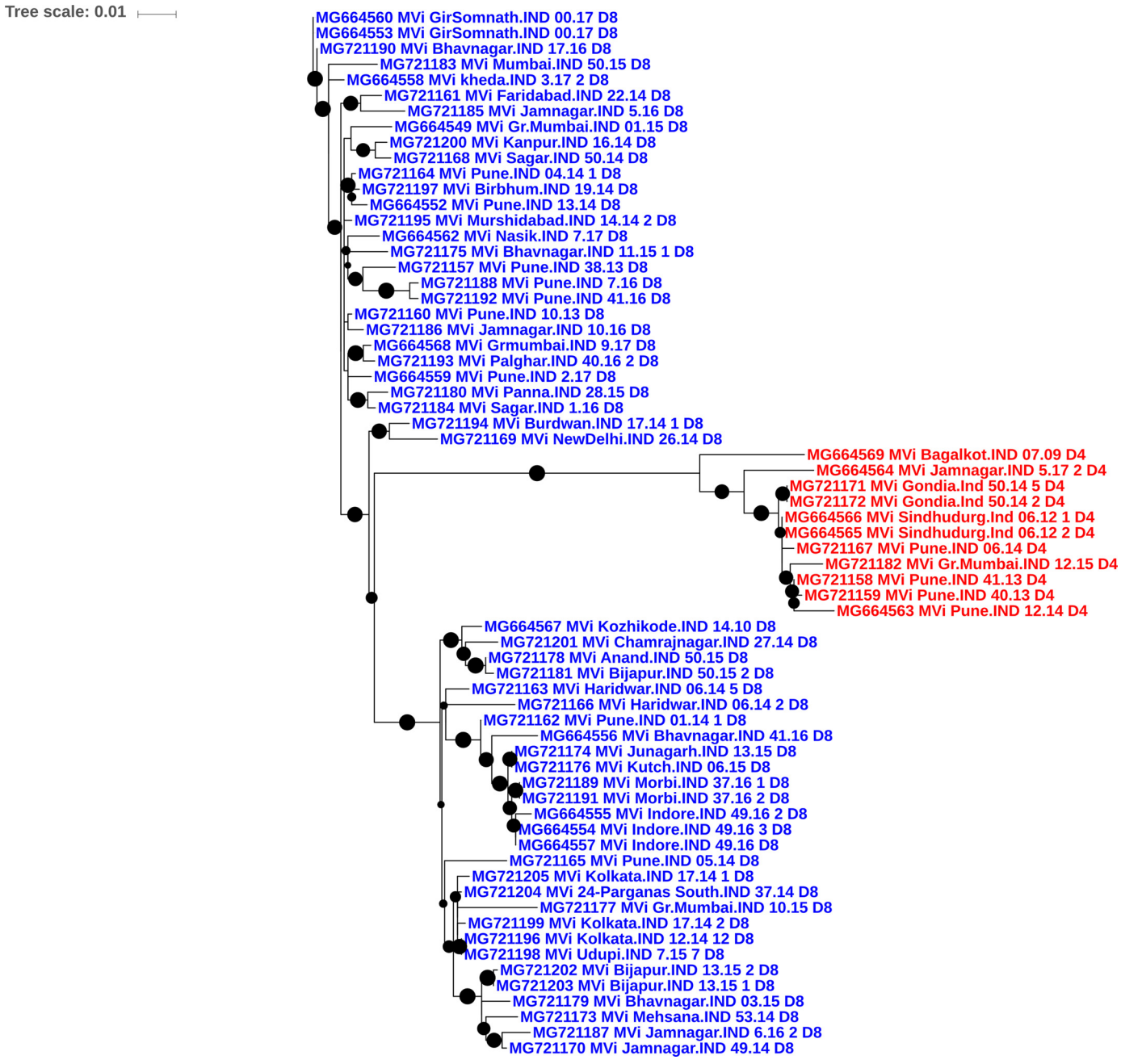

Fig. 1. Measles virus M/F region based phylogenetic tree. The Indian D8 highlighted in blue and D4 MeV isolates are highlighted in red colours.

mean PND was from 1.4 to 15.3%. The within and between genotypes mean PND for N gene was from 0.9% to 2.3% and 1.5% to 8%, respectively. For H gene the within and between genotypes mean PND was from 0.8% to 1.2% and 0.4% to 3.7%. Thus, non-overlapping ranges of PND within and between genotypes were observed for M/F gene as against overlapping ranges for N gene and H gene. Notably, the most heterogeneous genotype for M/F gene was D4 (mean PND within D4 was 3.35%), for N and H genes it was D8 (mean PND within D8 was 2.3% and 1.2%) respectively.

In the M/F non-coding region, all MeV genotype D4 isolates showed insertion of seven 'C' at 318–324 position resulted in length of 1019 nucleotides, whereas length of MeV genotype D8 isolates ($n = 56$) remain as 1012 nucleotides. All these D4 isolates with indels were obtained from the states of Maharashtra, Gujarat and Karnataka. Comparison with global MeV isolates revealed insertion at position 318–324 in MeV strains detected from Croatia, Italy, USA and Spain. In addition, deletions at different positions (620, 628 and 636) were noted in the MeV D4 isolates from Jamnagar and Sindhudurg districts. Deletions at positions 536 and 594 were noted in MeV D8 isolate from Indore district. In addition, insertion of 'C' at position 540 was noted in Indore-2016 D8 strain and insertion of 'CG' at position 956–57 in Pune-2013 D4 strain. Both insertions were noted in the part of 5′ UTR of F-gene. The multiple sequence alignment of M/F intergenic region reveals CCCCCCC and TCCCCCC inserts in Indian D4 isolates. Overall, three lineages of D4 isolates and five lineages of D8 isolates were noted.

The two-fold increase in the nucleotide substitution rate was noted for M/F compared to N and H genes. Overall, H and N genes contain up to 8% variability between different genotypes, however variability approaches to 12% for N gene.[2] The M/F region is considered as most variable region with 9.2% nucleotide variability, followed by 7.3% in N gene and 4.6% in H gene.[3]

For the first time, the sequencing of M/F region was successfully completed. Seven-nucleotide insertion in the matrix and a single nucleotide deletion in the fusion genes was documented. Present work concludes that true diversity of MeV in India, can be studied using M/F gene and further MeV transmission can be studied using identical inserts detected in the global isolates.

Ethical standard

This work has been approved by Institutional Ethical Committee for Humans, NIV Pune.

Conflicts of interest

The authors declare they have no conflicts of interest regarding this article. The research did not receive any specific grant from the funding agencies in the public, commercial, or not-for-profit sectors.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2019.05.011.

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A case of a FilmArray® ME false negative in meningococcal meningitis

Dear Editor,

Lumley and colleagues, in this Journal recently reported the enhanced utility of a multiplex PCR system in CNS infection.[1] Several real time PCR systems have been commercialized in recent years for diagnosing bacterial and viral meningitis and encephalitis.

Infectious meningitis and encephalitis are potentially life-threatening and require timely diagnosis and rapid initiation of effective antimicrobial therapy to avoid a fatal outcome or neurological damage. They can be caused by several pathogens, which often share similar clinical presentations. In bacterial meningitis the leading infectious agents are *Streptococcus pneumoniae, Neisseria meningitidis* and *Haemophilus influenzae*.[2]

Although cerebrospinal fluid (CSF) culture is the "gold standard" for meningitis diagnosis, molecular methods are now commonly used for the detection of central nervous system infection.[3] The FDA recently approved the first molecular panel for the diagnosis of meningitis/encephalitis. FilmArray® meningitis/encephalitis panel (BioFire, Salt Lake City, UT) detects the 14 most frequent pathogens causing meningitis and/or encephalitis, including 6 bacteria, 7 viruses, and 1 yeast.

We are reporting a case of a false negative FilmArray® result and how we managed to overcome this situation. A 36- year-old

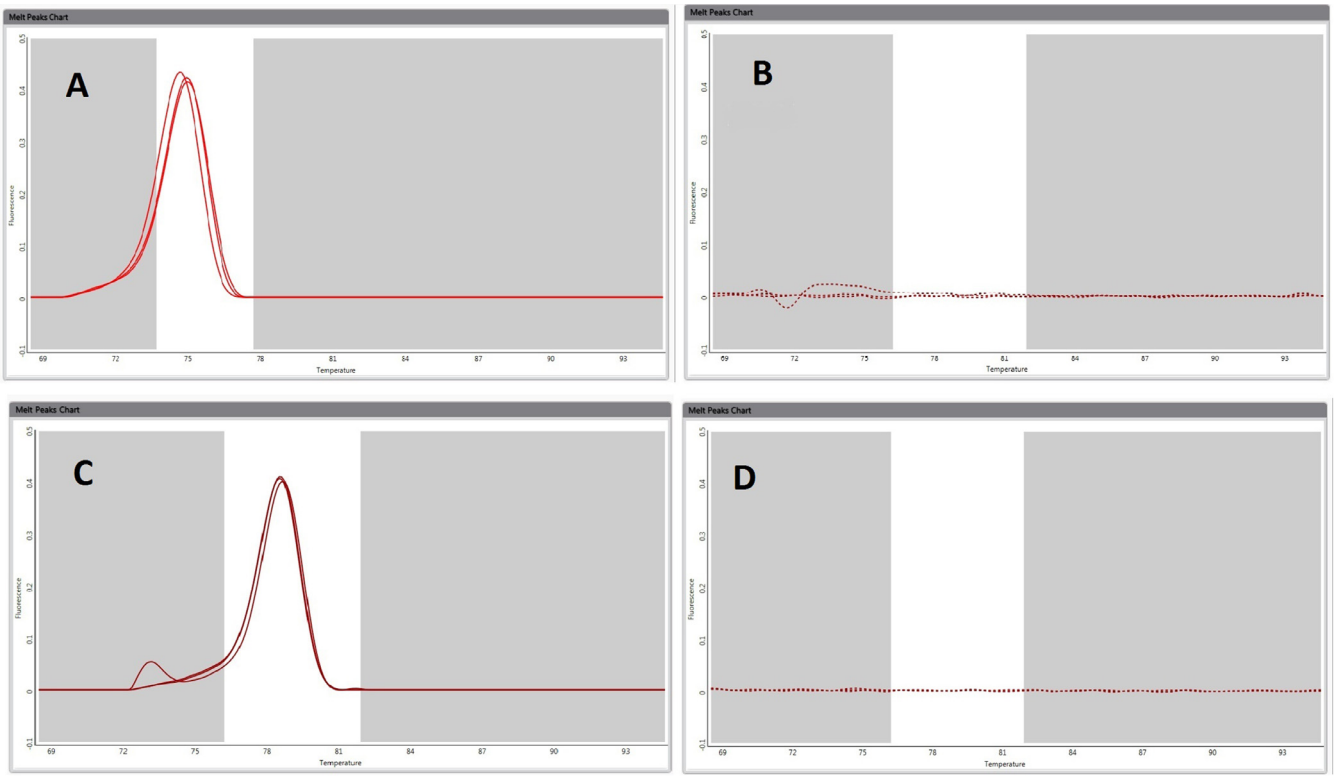

Fig. 1. (a) Internal control melting curves; (b) *Neisseria meningitidis* melting curves of the sample undiluted; (c) *Neisseria meningitidis* melting curves of the sample diluted 1/5; (d) *Neisseria meningitidis* melting curves of a negative sample.

man with no clinical history of interest was admitted to the emergency department after being found in his house not responding to stimulus. His wife reported that he had had an episode of confusion and 40 °C fever the day before. The patient received one dose of intravenous ceftriaxone (2 g) before lumbar puncture was performed. The CSF revealed a neutrophilic-predominant pleocytosis (white blood cell count 9200 cells/mm^3, 83% neutrophils), glucose 2 mg/dL [40–70 mg/dL], protein 641.3 mg/ dL [15–45 mg/dL] and lactate 18 mmol/L. Gram stain was negative and the FilmArray® test performed resulted negative. The FilmArray's internal control was reviewed showing amplification and no inhibition (Fig. 1). FilmArray® pouch contains two internal controls: an RNA process control that targets an RNA transcript from the yeast *Schizosaccharomyces pombe* and a PCR2 control that detects a DNA target dehydrated into the wells of the array.

Due the high suspicion of community-acquired bacterial meningitis all the pathogen's melting temperature curves were reviewed. A target-specific nested PCR and high-resolution melting are performed in three replicates. After completing the analytical run, the raw data is automatically interpreted by the on-board instrument software. At least two of the three melt curves must meet several analytical criteria in order for a target to be called as a positive detection. The *N. meningitidis* melting curves showed some alterations if it is compared with a negative sample for *N. meningitidis* (Fig. 1). Since the sample was highly purulent, we decided to repeat the test using a 1/5 dilution (sterile water) of the sample, resulting positive for *N. meningitidis* (Fig. 1). Routine cultures of CSF and blood were negative.

PCR can be inhibited by multiple substances.[4] Even though the internal controls did not showed inhibition we believe the sampleś high purulence and numerous leucocytes played a role in the failure of the *N. meningitidis* amplification results. This theory is supported by the difference between both results when the sample was diluted.

The cases of FilmArrayś false negative in bacterial meningitis are rarely described in the literature, although are more frequent in viral meningitis.[5] To our knowledge three cases of a film array false negative were reported, one of them is a false negative for *S.agalactiae*, the specimen was from *a* > 65-year-old male patient with an intrathecal device for drug delivery, the medical records indicated an infection in a pocket site, and the CSF parameters were all normal, with a single colony on the culture plates, the authors considered It was possible that this patient did not have true infectious meningitis but a device-related soft tissue infection.[3] In another study there were two potentially false-negative results by the FilmArray® ME panel, for *N. meningitidis* and *H. influenzae*. Both specimens had been stored at −70°C for more than three decades and the microorganisms had been never isolated in culture, the positive result was obtained by counter inmunoelectrophoresis (CEI). The authors considered that the original CIE result might have been misinterpreted or the target nucleic acid might have degraded during storage.[6]

In our case, considering the symptoms and sings highly suggestive of meningitis and the CSF biochemical decidedly compatible with bacterial meningitis, a FilmArray 's negative result was anomalous. Revising the melting curves and repeating the test with a diluted sample the positive result for *N. meningitis* was more suitable with the clinical data. It is very significant that the internal controls did not show something wrong about the test run. Our experience highlights the importance of clinical data when interpreting laboratory results and the need of monitoring the test results with the on board FilmArray® PCR Evaluator software by an accomplished microbiologist.

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