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Letter to the Editor

Candida infection of membrane oxygenator during ECMO therapy

Dear editor,

Recently, clinical characteristics and outcome of periprosthetic joint infections due to Candida species[1] and improvements of survival in candidemic patients by application of five different measures were published in the Journal.[2] We would like to contribute a clinical case with Candida albicans colonization and infection of the extracorporeal Membrane Oxygenator (MO) inducing failure of the Extracorporeal Membrane Oxygenation (ECMO) which underlines the difficulties in foreign body associated Candida infections. To the best of our knowledge, this is the first study able to assign the cause of Candidemia clearly to the extracorporeal membrane oxygenator (MO) using a modified diagnostic approach broadening the spectrum of Candia associated infections.

Here, we present a case of a 58-year-old man who was admitted to the Intensive Care Unit of the Medical University of Graz, Austria with community-acquired pneumonia due to *Legionella pneumophila*. Acute respiratory distress syndrome necessitated intubation and controlled invasive mechanical ventilation with FiO_2 of 100% immediately after admittance to the Intensive Care Unit. Nevertheless, the pO_2/FiO_2 ratio was 52–59 mmHg. Therefore, we provided ECMO through cannulation of the right femoral and right internal jugular vein (iLA Activve, Novalung, Heilbronn, Germany) rendering possible lung protective ventilation. Sequential cultivation of blood specimen and bronchoalveolar lavage on day 1 and on day 7 did not reveal any other infectious agent than *Legionella pneumophila,* as DNA of *Legionella* species was detected by PCR in samples of two distinct bronchoalveolar lavages. We started continuous veno-venous hemodialysis with regional citrate anticoagulation (MultiFiltrate Ci-Ca, Fresenius, Bad Homburg, Germany) due to acute kidney injury and high-dose norepinephrine due to septic shock. C-reactive protein reached a nadir on day 7 (147 mg/dL, normal < 5), of fluoroquinolone treatment. After 11 days of ECMO therapy, the patient developed severe septic shock with necroses of several toes and digits on both hands. Multiple whitish spots appeared on the MO (Fig. 1A). In parallel, the inflow (arterial) line pressure of the membrane increased leading ultimately to cessation of extracorporeal circulation. The patient died on the same day. Blood cultures drawn simultaneously from the central venous catheter inserted in the left internal jugular vein, where the tip of the catheter was close to the tip of the outflow line of the MO, and a peripheral vein prior death revealed *Candida albicans* bloodstream infection with a differential time to positivity of 2 hours (25.3 h hub blood culture vs. 27.3 h peripheral blood culture). Moreover, *post mortem* analysis of the MO revealed extensive colonization with *Candida albicans* that had led to failure of extracorporeal circulation and oxygenation, and ultimately death. Blood was obtained from inflow line and the outflow line of the MO, 100 µL each was plated onto chocolate agar and incubated at 35 °C. Colonies were read after 24 and 48 h. While no colonies were detected in the sample from the inflow line (Fig. 1B), a high load of *Candida albicans* (10^4 colony forming units (CFU)/mL) was found in the outflow line of the MO reintroducing oxygenated blood to the patient (Fig. 1C). This finding indicated not only colonization, but obviously extensive amplification of *Candida albicans* fungal burden in the MO representing a new subentity of ECMO Device Related Bloodstream Infections (EDR-BSI). Confirming these results a sophisticated diagnostic approach using 'histopathological' examination of the artificial hollow fiber membrane of the MO showed a high amount of branching *Candida* hyphae with positive reaction in Periodic-acid Schiff staining (Fig. 1D). *Candida albicans* identification in histopathological slides was performed by specific peptide nucleic acid fluorescent in situ hybridization (AdvanDx, Woburn, MA).

Previous studies have shown that patients undergoing ECMO therapy face a higher risk of invasive candidiasis.[3,4] Our case report highlights this issue, and focuses on the intrinsic function of the MO as a reservoir and possible multiplier for nosocomial superinfection with *Candida albicans*, which was apparently shielded from reaction of the immune system by the polymethylpentene membrane. While most candidemia cases are endogenous in origin, the source of candidemia in our patient remains unclear.[5] Contamination of the MO during initial installation (e.g. *via* contaminated cannulation) seems unlikely given the long incubation period of 11 days after introduction of active lung assistance. Although differential time to positivity was consistent with definitions of catheter-related bloodstream infection using differential time to positivity method, the high fungal burden in the outflow line of the MO entering the right internal jugular vein might have locally increased the fungal burden of hub blood cultures drawn from the contralateral left internal jugular venous catheter.[6] Negative inflow cultures might have resulted from the low systemic fungal burden in peripheral blood as indicated by the time to positivity of 27.3 h, which corresponds to approximately 10 CFU/mL *Candida* in venous blood.[7] Culture of only 100 µL of inflow line blood on chocolate agar might therefore miss the fungal burden of 10 CFU/mL. Regardless of the initial source of candidemia, *Candida* colonization of the MO at least perpetuated infection. Thus, *Candida albicans* infection of the oxygenation device causing a new subentity of EDR-BSI, here reported for the first time by using a more sophisticated diagnostic approach, represents a severe complication of active extracorporeal lung assistance. It can be fatal and must be immediately recognized and treated with prompt change of the membrane oxygenator accompanied by intravenous antifungal therapy. In the context of current literature and consideration of our findings we suggest to discuss additional measures for ECMO monitoring, diagnosis of ER-BSI and ERBSI treatment. Daily macro-

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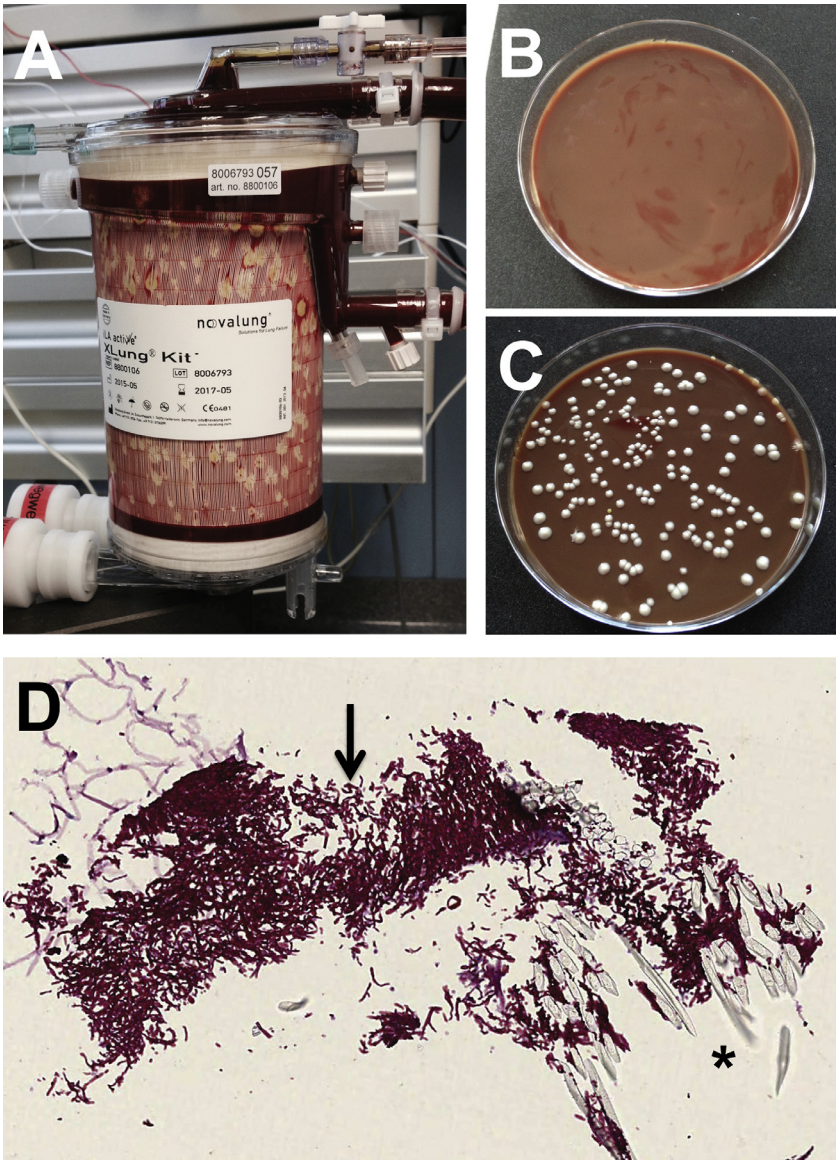

Fig. 1. Extracorporeal membrane oxygenator overgrown with *Candida albicans.*
A) The multiple whitish spots on the extracorporeal membrane oxygenator were due to extensive colonization with *Candida albicans*. Blood samples from the inflow and outflow lines of the extracorporeal membrane oxygenator were obtained and 100 µL each plated onto chocolate agar. While no colonies were detected from the inflow line (**B**), a high load of *Candida albicans* (**C**) was found in the outflow line reintroducing oxygenated blood to the patient. Histological section of the extracorporeal membrane oxygenator (**D**) showed massive accumulation of Periodic-Acid Schiff-positive branching *Candida* hyphae (arrow) between the crystalline-looking polymethylpentene membrane particles (asterisk) (magnification x20).

scopic exploration of the MO to identify surface alterations such as whitish spots, sampling of blood cultures simultaneously pre- and post-MO for better differentiation towards the source of infection and consideration of not only MO thrombosis but also Candida overgrowth within the MO if perfusion pressure across the MO rises.

Ethical standard

The study was approved by the institutional review board of the Medical University of Graz, Austria (# 28-176 ex 15/16).

Conflicts of interest

The authors declare that there are no conflicts of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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The advantages of next-generation sequencing technology in the detection of different sources of abscess

Abbreviation

NGS	next-generation sequencing
CNS	central nervous system
CRP	C-reactive protein
MRI	magnetic resonance imaging
ER	emergency room
CSF	cerebrospinal fluid

Dear Editor,

Ai et al.,[1] in this Journal, held the view that the comprehensive diagnostic ability of Next-generation Sequencing (NGS) in identifying pathogens of unknown etiology during central nervous system (CNS) infection and displayed the possible potential that NGS may hold by reflecting disease progression through its direct and semi-quantitive surveillance of pathogen loads. We completely agree with the opinion that rapid and accurate diagnosis of causative agents in infectious disease remains challenging, and NGS, as a diagnostic approach, may provide a promising comprehensive ability for the identification of pathogenic microorganisms from patients. Here we present a group of individual cases with different sources of abscess, where NGS was used for pathogenic confirmation.

Case 1, a one-year-old previously healthy girl was sent to local emergency room (ER) for intermittent fever and the discovery of waist mass with pus outflow. Her blood routine test showed increase white blood cell count (23.77×10^9/L), with 76.1% granulocytes, C-reactive protein (CRP) was over than 160 mg/L. The magnetic resonance imaging (MRI) showed thick-walled lesion in front and both sides of the vertebral body from the 2nd lumbar vertebrae to the caudal vertebrae, considering infection, malformation or tumor (Fig. 1A). Ceftriaxone and meropenem were prescribed, but failed to relieve the symptoms.

Case 2, a 2-month-old infant was sent to local ER for continuous fever and weakness, his brain MRI showed the circular abnormal signal in the left frontal lobe (Fig. 1B). His blood routine test showed a white blood cell count of 24.44×10^9/L, with 40.4% granulocytes, CRP was 47.8 mg/L. Ceftriaxone, vancomycin and mannitol were prescribed, but failed to relieve the symptoms.

Case 3, a 6-year-old girl went to ER because of continuous fever, convulsion and swelling of right eyelid. Orbit and sinus CT indicate soft tissue swelling in the right orbit and periorbit, right orbital cellulitis (Fig. 1C). His cerebrospinal fluid (CSF) examinations revealed increased leucocyte count (22×10^6 cells/L) with normal glucose and protein level, while cranial MRI showed abnormal meningeal enhancement. Empirical antibiotic treatment including intravenous ceftriaxone, cefoperazone-sulbactam were administered but the patient's symptoms did not relieved.

Case 4, a one-year-old previously heathy child went to local ER because of continuous fever and left lateral cervical masses. His cervical B-ultrasound revealed multiple enlarged lymph nodes in the left cervical region. An initial laboratory evaluation showed a white blood cell count of 19.26×10^9/L, with 6% granulocytes, 81.9% lymphocytes. CRP level increased (80 mg/L), as well as procalcitonin (1.53 ng/ml). Empirical antibiotic treatment including intravenous ceftriaxone, ertapenem were administered but the patient's fever and cervical masses did not relieve.

Case 5, a 4-year-old previously heathy boy went to outpatient department for intermittent fever with cervical masses. His blood routine test is normal but has a markedly increased blood CRP (25 mg/L). Ultrasound of the neck soft tissue revealed a left submandibular cystic mass with a range of approximately 2.5 cm × 2.7 cm × 2.6 cm. The fluid was turbid. Ceftriaxone was prescribed. However, the child still had intermittent fever and pronounced cervical masses.

All 5 patients were then transferred to our hospital, NGS and culture of the different sources of abscess were performed. Pathogenic information of the abscess sample from five patients are summarized in Fig. 1 and Table 1. NGS detected *Streptococcus anginosus, Escherichia coli* and *Bacteroides salanitronis* in patient 1, *Bacteroides fragilis, Staphylococcus aureus, Streptococcus pyogenes* and *Haemophilus influenza* in patients 2–5, respectively, consistent with the simultaneous culture results in patients 1–3.

With the NGS detection, all the five patients were treated with the appropriate antibiotic treatment. For patient 1, we did the 2nd puncture after one months of antibiotic therapy, and NGS was re-performed with a markedly decrease sequencing reads (5) of *Streptococcus anginosus* and no more sequencing reads of *Escherichia coli*. For patient 5, the patient got the positive culture of *Haemophilus influenza* at the 2nd puncture 6 months later for the recurrence of cervical lymphadenitis. He was finally diagnosed as thyroglossal tract cyst through operation. All the patients were completely recovered after all.

Currently, NGS has been successfully applied to investigate the etiology of infectious diseases, e.g. central nervous system,[2,3] respiratory system[4] and blood,[5] as well as bone and joint infection.[6] However, NGS using in the field of abscess is really rare. Kuroda et al. reported a case that deep sequencing detected *Francisella tularensis* in the pus sample from the axillary abscess.[7] Gong et al. presented a patient with *Klebsiella pneumoniae* liver abscess detecting by nanopore sequencing.[8] In this study, we performed metagenome analysis by NGS on different sources of abscess from five patients. For the cases of having positive culture results, the metagenome analysis results were all consistent with those of conventional culture examination. It often takes 3–5 days for conventional clinical microbiology to reach final pathogen confirmation.[9] And delay in pathogen determination may lead to improper antibiotics therapy and finally higher mortality rates among patients with severe infection. Hence, the appropriate treatment process according to the pathogen result could be rapidly determined by NGS analysis than ever before.

In addition, a wide range of microorganisms can cause abscess. Taking full advantage of unbiased sequencing, NGS could detect multiple pathogenic microorganisms in prior study.[10] This strengthen was once again confirmed by our study among patients with abscess patients.

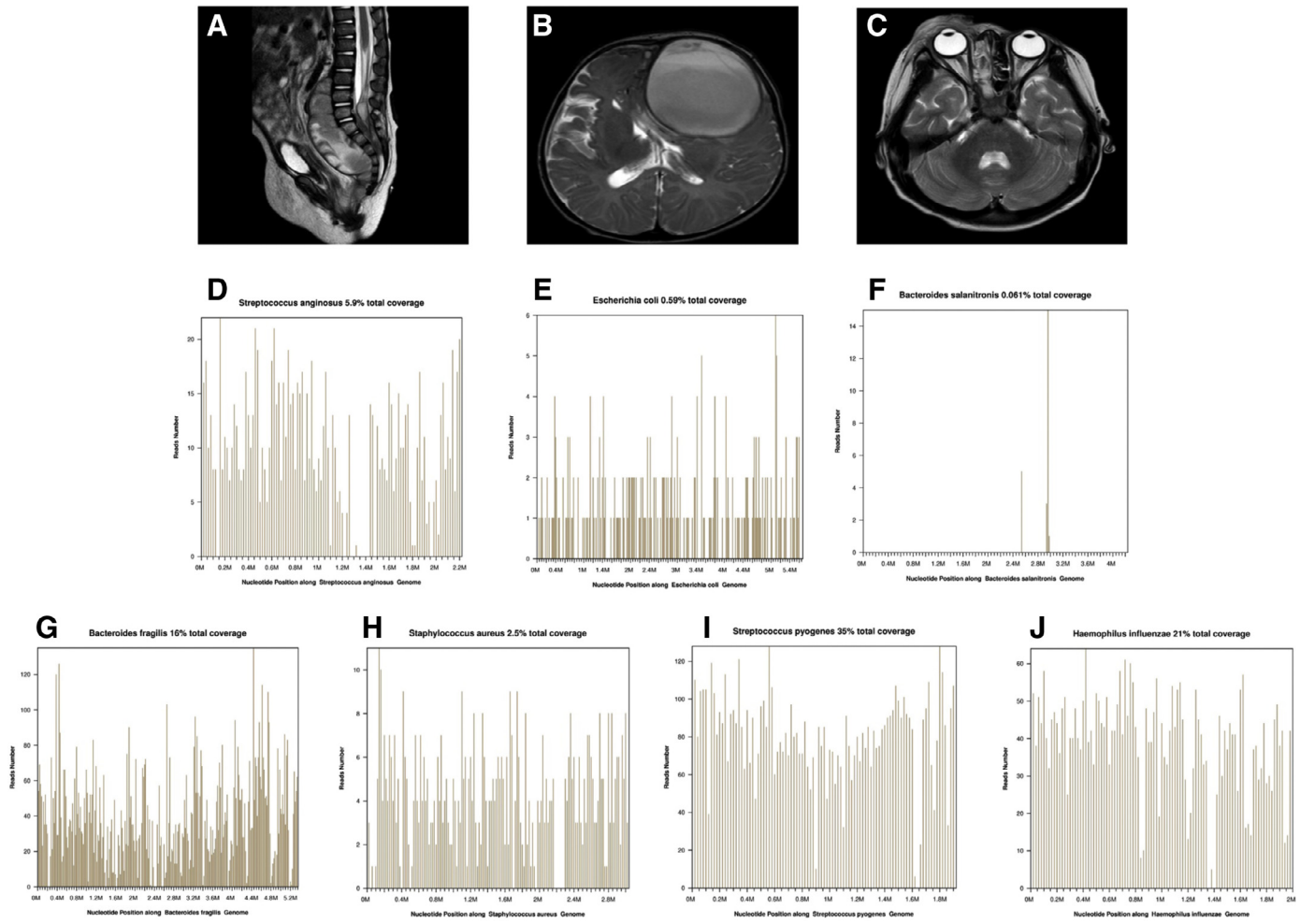

Fig. 1. MRI findings and NGS information of different patients. A sagittal T2-weighted image of sacrococcygeal region revealed thick-walled lesion in front and both sides of the vertebral body from the 2nd lumbar vertebrae to the caudal vertebrae, lakes signals in vertebral canal from the 4th lumbar vertebrae to the 2nd sacral vertebrae and spinal dermal sinus tract in the 3rd sacral vertebrae (Panel A). An axial T2-weighted image of cranial MRI revealed the circular abnormal signal in the left frontal lobe with liquid plane in the cyst and compressive displacement of midline structure, bilateral frontal lobes and lateral ventricle (Panel B). An axial T2-weighted image of cranial MRI revealed thickened right eyelid, external protrusion of the right eyeball, long T2 signal shadow in the right eye orbit and right paranasal sinus group (Panel C). Panels D–J showed the NGS information of the different sources of abscess samples from five patients. Panel D to F showed the results of patient 1 and Panels G–J showed patients 2–5, respectively.

Table 1
Pathogenic information of the abscess samples from five patients who developed different source of abscess.

No.	Age	Source of abscess	NGS results		Culture results
			Bacteria (reads)	Virus/Fungi/Parasite	
1	1-year-old	Abdominal abscess	*Streptococcus anginosus (883)* *Streptococcus constellatus (97)* *Escherichia coli (23)* *Escherichia fergusonii (14)* *Bacteroides salanitronis (17)* *Shigella dysenteriae (15)* *Shigella sonnei (14)* *Prevotella intermedia (28)* *Prevotella melaninogenica (16)* *Staphylococcus epidermidis (70)*	None	*Streptococcus anginosus* *Escherichia coli*
2	2-month-old	Brain abscess	*Bacteroides fragilis (5165)* *Bacteroides xylanisolvens (131)* *Alistipes shahii (9)* *Alistipes finegoldii (3)* *Odoribacter planchnicus (18)* *Porphyromonas gingivalis (4)*	None	*Bacteroides fragilis*
3	6-year-old	Orbital abscess	*Staphylococcus aureus (543)* *Klebsiella (13)*	None	*Staphylococcus aureus*

(*continued on next page*)

Table 1 (*continued*)

No.	Age	Source of abscess	NGS results		Culture results
			Bacteria (reads)	Virus/Fungi/Parasite	
4	1-year-old	Cervical lymphadenitis	*Streptococcus pyogenes (2858)* *Streptococcus equi (74)* *Clostridium luyveri (3)*	None	*None*
5	4-year-old	Cervical lymphadenitis	*Haemophilus influenza (9585)* *Haemophilus parainfluenza (45)* *Pasteurella multocida (42)* *Aggregatibacter aphrophilus (13)* *Neisseria meningitidis (5)* *Burkholderia (8)*	None	*Haemophilus influenza*

Prompt identification of microorganism is critical for not only definite treatment, but also infection surveillance. One patient in our study did two times NGS detection in acute and recovery phase which demonstrated dramatically decrease reads of identification pathogens, displaying the possible capacity that NGS may have the potential to be an encouraging tool by reflecting disease states through its semi-quantitive surveillance of pathogen loads. These results are consistent with Ai's data.[1]

Here, we originally reported the applcation of NGS to identify different sources of abscess pathogens indicating this technology provides a powerful ability for the rapid etiology diagnosis of patients with abscess and is expected to be one of the most valuable, supplemental tool in clinical diagnostic microbiology by offering unbiased sequencing and culture-free infections pathogen detection.

Declaration

We confirm that each individual named as an author meets the journal's criteria for authorship and neither the entire paper nor any part of its content has been published or accepted elsewhere. It is not being submitted to any other journal.

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Conflict of interest

All authors: No potential conflicts of interest.

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Diagnostic stewardship in the post-vaccine era: Reducing demand for meningococcal and pneumococcal PCR

Dear Editor,

We read with interest the article by Zheng et al. published in May 2018 (Meningococcal disease and control in China: Findings and updates from the Global Meningococcal Initiative), which describes the changing vaccine-associated epidemiology of meningococcal disease in China and the use of polymerase chain reaction

(PCR) in the diagnosis of meningococcal disease.[1] PCR is considered one of the greatest discoveries in molecular biology and the use of multiplex PCR panels maximises laboratory efficiency and productivity, but necessitates diagnostic stewardship.[2,3,4,5] Definitions of diagnostic stewardship vary but most authors agree that it refers to the appropriate use of laboratory testing to guide patient management in order to improve clinical outcomes.[6,7] Sir William Osler (1849–1919) famously said that *"medicine is a science of uncertainty and an art of probability"* and keeping pre-test probability to the fore when selecting PCR tests is an essential learning point for microbiology laboratory users to ensure minimal false-positive results, avoid unnecessary over-testing and identify true positive results.[8,9]

With diagnostic stewardship in mind, we recently reviewed 115,620 PCR specimens processed (1999–2017) at the Irish Meningococcal and Sepsis Reference Laboratory (IMSRL), Dublin, Ireland which provides a government-funded diagnostic bacterial PCR service (blood EDTA, cerebrospinal fluid (CSF), pleural fluid, joint fluid, pus). The aims of this review were to assess the impact of a switch to syndromic PCR ordering for laboratory service users and to evaluate the impact on the number of samples processed (blood EDTA and CSF) as a consequence of established vaccination programmes against *Streptococcus pneumoniae, Haemophilus influenzae* type B (Hib) and *Neisseria meningitidis* serogroups C and B. Other sterile site fluids were excluded from our analysis as they were more recently introduced and long-term data are not yet available.

Between 1999 and 2002, blood and CSF PCR testing for *N. meningitidis* only was provided at the IMSRL. Molecular diagnostics for *S. pneumoniae* and Hib commenced in 2003 (in 2013 routine PCR for Hib was replaced with an assay that detects all *H. influenzae*). The public health benefits from the provision of childhood vaccines targeting Hib (introduced 1992), *S. pneumoniae* (PCV7 introduced into the routine primary immunisation schedule in September 2008, PCV13 vaccine replaced PCV7 in 2010), meningococcal serotype C (introduced 2000) and serotype B (introduced 2016) is demonstrated in Fig. 1(a) with a steady reduction in the number of PCR-positive specimens, though a large proportion of this reduction is related to a natural decline in the circulation of virulent meningococcal serotype B subtypes. In 2000, 3857 blood EDTA samples and 1009 CSF samples were processed using meningococcal PCR, of which 333 (8.6%) and 92 (9.1%) were positive, respectively. This is in contrast to 2017 meningococcal data where 1932 blood EDTA samples and 1173 CSF samples were processed with 43 (2.2%) of blood EDTA specimens and 21 (1.8%) CSF specimens authorised as positive. Following the introduction of syndromic testing in 2015, there has been a reduction in the total number of specimens processed annually at the IMSRL as shown in Fig. 1(b); 11,582 specimens received in 2014 compared with 4996 in 2017.

A switch to syndromic testing was introduced with the clinical objective of assisting laboratory users to remain focussed on sending samples for processing for high probability bacterial pathogens in keeping with the patient's presenting complaint (Table 1).[10] Site visits to over half of the top 15 service user hospitals around Ireland were performed between 2014 and 2015, delivering an interactive presentation regarding the proposed PCR service; for example, where a working diagnosis of osteomyelitis exists, first-line PCR testing is for *Staphylococcus aureus*, S. *pneumoniae* and Group A *Streptococcus* but PCR testing for other pathogens is only available on request following consultation with a Consultant Microbiologist either at the beside (if patient is admitted to our own hospital co-located with the IMSRL) or a telephone consultation (if patient is in a referring hospital). At a national level, liaison with the Department of Public Health and the Health Protection Surveillance

Table 1
Current selection criteria for PCR testing at the Irish Meningococcal and Sepsis Reference Laboratory (IMSRL), Dublin, Ireland.

Syndrome	Sample for PCR	GBS^	*Escherichia coli*	*Neisseria meningitidis*	*Streptococcus pneumoniae*	*Haemophilus influenzae*	*Staphylococcus aureus*	GAS*	*Kingella kingae*
Meningitis (≥ 7 days of life)	CSF[%]	Only if aged < 90 days	Only if *E. coli* bacteraemia or UTI and aged < 90 days or evidence of meningitis or has galactosaemia	All	All	Only if high CSF white cell count noted on the request form	Special request	Special request	Special request
Sepsis (≥ 7 days of life)	Blood	Special request	Special request	All	Only if radiographic evidence of pneumonia	PICU[$] patients	Special request	Special request	Special request
Early-onset sepsis & meningitis (< 7 days of life)	Blood CSF	All	Special request	Special request	Special request	Special request	Special request	Special request	Special request
Complicated pneumonia - parapneumonic effusion	Pleural fluid	Special request	Special request	Special request	All	Special request	Special request	Special request	Special request
Osteomyelitis/septic arthritis	Joint fluid	Special request	Special request	Special request	All	Special request	All	All	Only if aged < 5 years

[%] CSF: cerebrospinal fluid.
^ GBS: Group B *Streptococcus*.
* GAS: Group A *Streptococcus*.
$ PICU: Paediatric Intensive Care Unit.

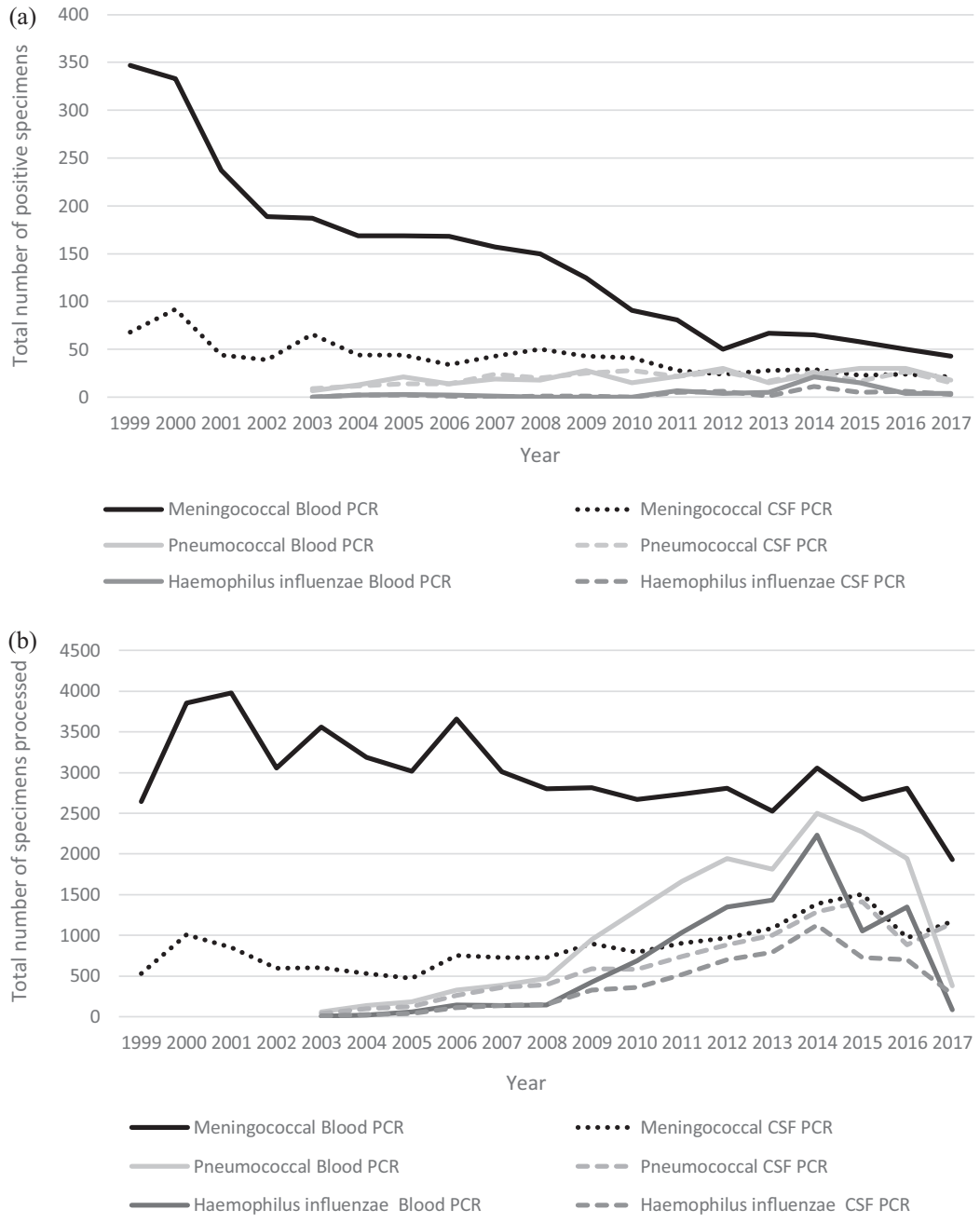

Fig. 1. **(a)** Total number of positive specimens 1999–2017 from adult and paediatric hospitals in Ireland ($n = 4234$); meningococcal blood PCR positive $n = 2736$, meningococcal CSF PCR positive $n = 785$, pneumococcal blood PCR positive $n = 304$, pneumococcal CSF PCR positive $n = 297$, *Haemophilus influenzae* blood PCR positive $n = 68$, *Haemophilus influenzae* CSF PCR positive $n = 44$. **(b)** Total number of specimens processed 1999–2017 from adult and paediatric hospitals in Ireland ($n = 115{,}620$); meningococcal blood PCR processed $n = 56{,}787$, meningococcal CSF PCR processed $n = 16{,}490$, pneumococcal blood PCR processed $n = 16{,}334$, pneumococcal CSF PCR processed $n = 9826$, *Haemophilus influenzae* blood PCR processed $n = 10{,}165$, *Haemophilus influenzae* CSF PCR processed $n = 6018$.

Centre, Dublin was necessary to ensure rapid and appropriate responses to changes in the levels of detection of pathogens associated with invasive infection.

Clinical microbiologists have always provided diagnostic advice on pathogenic microorganisms with the inherent objective of improving clinical outcomes for patients by recommending appropriate diagnostic tests. The same principles still applies in the post-vaccine era with regard to PCR. Molecular diagnostics will continue to evolve further challenging microbiologists to manage the ordering of molecular investigations carefully in the clinical context of the patient and taking account of vaccine-associated changes in disease epidemiology.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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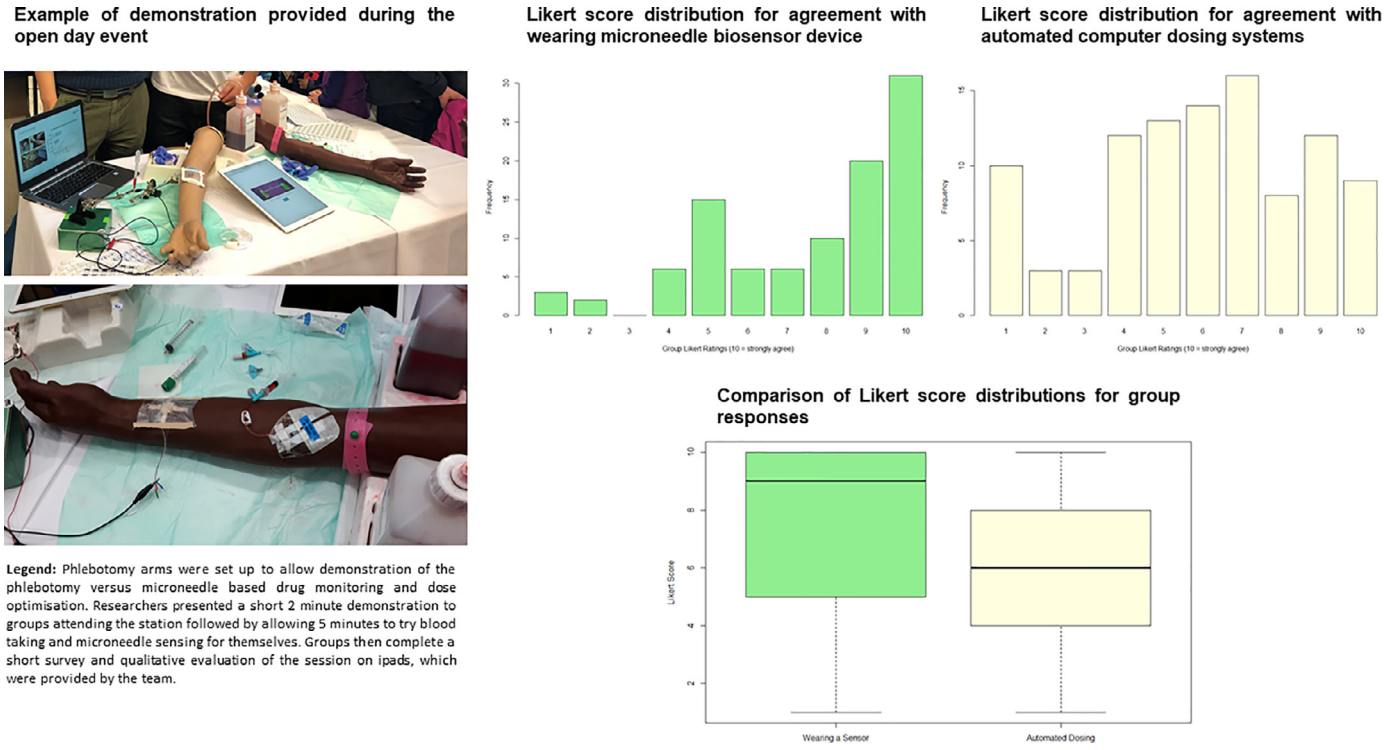
Public acceptability of computer-controlled antibiotic management: An exploration of automated dosing and opportunities for implementation

Dear Editor,

We read with interest the article by Pan and colleagues on the role of aptamers in infectious diseases.[1] As well as diagnostic and drug delivery, aptamers have a potential role for facilitating the real-time monitoring of antimicrobial therapy. Within healthcare there is a strong emphasis on the development and introduction of novel technologies, including those that make automated, computer-controlled decisions.[2] These technologies offer the potential to enhance the precision with which we practice medicine. However, there are also concerns surrounding the safety of such devices, especially when human decision making is removed from their context.[3] Whilst there is now a healthy debate on the subject of automated, intelligent technologies in the literature and media, there remains a paucity of work exploring citizen views on the acceptability of such intervention.[4,5]

Public festivals offer the opportunity to rapidly collect and explore citizen views on focused subjects, having successfully been used by our group to explore a number of infection related topics.[6,7] Within this study, we explored citizen perceptions of using microneedle-based biosensors and computer-controlled dose optimisation software for the delivery of precision antibiotic dosing. Microneedle-based technology is rapidly expanding, with *in-vivo* clinical studies of these devices underway for monitoring a range of molecules, including antibiotics.[8]

This study was performed across two days (28th and 29th April 2018) at a London university public festival. The festival is open to the public and is visited by over 15,000 people annually. A stand was set up in the "infection zone" (which is visited by > 3,000 people) of the festival (Fig. 1). Two phlebotomy arms were set up to demonstrate traditional phlebotomy versus the use of microneedle technology for continuous antibiotic monitoring.[9,10] Over the festival, two researchers (TMR and DM) manned the scenario which was visited by groups of 2–6 people for 10-min periods. During visits, the group had a 1-min demonstration of traditional drug monitoring versus the use of microneedle technology on the phlebotomy arms. A simulation of a closed-loop control system for computerised antibiotic dose optimisation using the microneedle technology was then demonstrated. The groups then had the remaining time to attempt phlebotomy and use the microneedles on the demonstration arms and complete a short survey (**Appendix 1**) providing one set of answers (agreed as a consensus). This methodology was the same on both days except for



Legend: Phlebotomy arms were set up to allow demonstration of the phlebotomy versus microneedle based drug monitoring and dose optimisation. Researchers presented a short 2 minute demonstration to groups attending the station followed by allowing 5 minutes to try blood taking and microneedle sensing for themselves. Groups then complete a short survey and qualitative evaluation of the session on ipads, which were provided by the team.

Fig. 1. Example of demonstration and Likert score summaries of group acceptance of demonstrated technologies at a public festival.

the questions asked. On day two, individual groups were asked to undertake an additional task to facilitate triangulation of findings from day one. Groups undertook discussion to agree or disagree with the answers obtained from day one and provided comments. For speed and anonymity, group demographics were not collected. All data were collected electronically, using a free data entry interface (typeform.com) and a tablet device. All quantitative responses were analysed in R. Qualitative responses were analysed using line-by-line coding undertaken independently by two researchers (TMR and DM) to group responses into common categories and then themes. This project was reviewed by the regional ethics committee, who deemed that given the anonymous nature of data collection, ethical approval was not required. Participants providing anonymous votes and written justifications at the festival were not required to provide written consent.

In total, the stall was visited by 100 groups (56 /100 day one and 44/100 day two). Median (range) group size was 4 (2–6) people. Groups spent a mean (SD) of 7 (3) min discussing and responding to the questionnaire after the short demonstration. Overall, the groups demonstrated good knowledge regarding the importance of antibiotic dose optimisation. On day one, 47/57 (82%) of groups identified that individuals need differing doses of antibiotics to treat their infections. The majority believed that antibiotic monitoring was beneficial for improving treatment of infections, stopping the development of drug resistance, and preventing side effects (36/57; 64%). The majority of individuals believed that their doctor should be the individual who decides what dose of antibiotic is delivered (35/57; 63%), followed by a decision from a computer-controlled programme (15/57; 27%). This was corroborated by participating groups on day two (34/44; 77%) with a high level of agreement for the use of microneedles for antibiotic monitoring (40/44; 93%).

Fig. 1 summarises the groups reported agreement with the use of microneedle-based technology to monitor antibiotic concentrations and automated computer-controlled dosing, respectively. These responses used a Likert scale from 1 to 10, where 10 was strong agreement with the statement. These results demonstrated high agreement with use of microneedle technology scoring a median (IQR) of 9/10 (5–10) with the groups having less confidence in automated dosing systems, scoring a median (IQR) of 6/10 (4–8).

Qualitative analysis of the group responses demonstrated common themes driving the agreement with the use of microneedles. These were that the microneedles would be less intrusive and less painful than blood testing. Furthermore, the groups felt that microneedles may also improve the accuracy and therefore effectiveness of antibiotic treatment. However, concerns were also noted about potential errors in the sensor technology and its impact on day-to-day activities, such as showering, if worn for prolonged periods of time. In contrast to sensors, there was a broader range of opinions reported with regards to automated dosing systems. On one hand, citizens believed that computers may be safer and less prone to mistakes compared to humans. However, citizens also reported concerns over the use of unsupervised systems, stating that they would prefer trained humans to be the final decision makers. This was because they believe that humans can contextualise the decisions being made, helping to guide more individualised and humanised decisions on dosing.

Although this study was limited by its small sample size, lack of demographic data, and potential for citizens attending to have a favouring view of science; it demonstrates that citizens are willing to accept the use of novel technologies, including those using computer-controlled decisions. However, there are concerns over the unsupervised nature of such systems, with the need for recommendations to be contextualised by a human still favoured. Future work must consider the greater role of citizen engagement in the development of such technologies, to ensure their acceptability upon implementation in clinical practice.

Contribution statement

TMR developed the idea for this study. All authors contributed significantly towards the development of the methodology and

demonstration performed during the study. TMR and DM undertook data collection and primary analysis. All authors contributed to the analysis and finalisation of data. TMR drafted the initial draft of the manuscript with all authors significantly contributing to the development and finalisation of the final iteration for submission.

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Conflict of interest

The authors have no conflicts of interest to declare.

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Supplementary materials

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Emergence of Coxsackie A6 hand-foot-and-mouth disease and comparative severity of Coxsackie B vs. echovirus infections, 2014–2016, UK

Dear Editor,

We note with interest the high background seropositivity of EVD68 in infants and children in some parts of China (Jiangsu).[1] Similarly, in our study below, we only found one acute case of EVD68 in our patient cohort, suggesting that most paediatric and adult EVD68 infections cause asymptomatic or mild disease.

Enterovirus D68 is one of many enteroviruses (EVs) belonging to the Picornavirus family. These are non-enveloped, single-stranded, positive-sense RNA viruses, with over 100 serotypes. They can cause a wide variety of paediatric and adult infections, including neonatal sepsis, viral (aseptic) meningitis and other neurological disorders, and hand-foot-and-mouth-disease (HFMD).[2-4] We present a 3-year (2014-2016) analysis of enteroviruses circulating in a UK Midlands paediatric and adult patient population, using both clinical and viral typing data.

All EV-positive cerebrospinal fluid (CSF) samples, which were tested as part of routine clinical work-up for paediatric sepsis

Table 1
Clinical parameters analysed to compare the severity of illness in patients infected with Coxsackie B versus echoviruses.

Parameters		Coxsackievirus B		Echovirus	p-value
Age (year)	n	Median (Q1–Q3) or %	n	Median (Q1–Q3) or %	
0–1	12/15	80%	31/60	52%	0.1412
2–19	0/15	0%	6/60	10%	
20–68	3/15	20%	23/60	38%	
LOS (days)	15	4 (3-6)	60	3 (2-4)	**0.0325**
CRP	15	5 (3-9)	59	14 (3-28)	**0.0195**
Lymphocytes (blood)	15	5.44 (2.44-7.51)	57	2.36 (1.23-3.9)	**0.0105**
CSF protein	15	0.64 (0.43–0.98)	58	0.435 (0.38–0.59)	**0.0283**
CSF WCC	15	103 (75–417)	57	29 (4–97)	**0.0085**

LOS - length of stay; CRP - C-reactive protein; CSF - cerebrospinal fluid; WCC - white cell count.

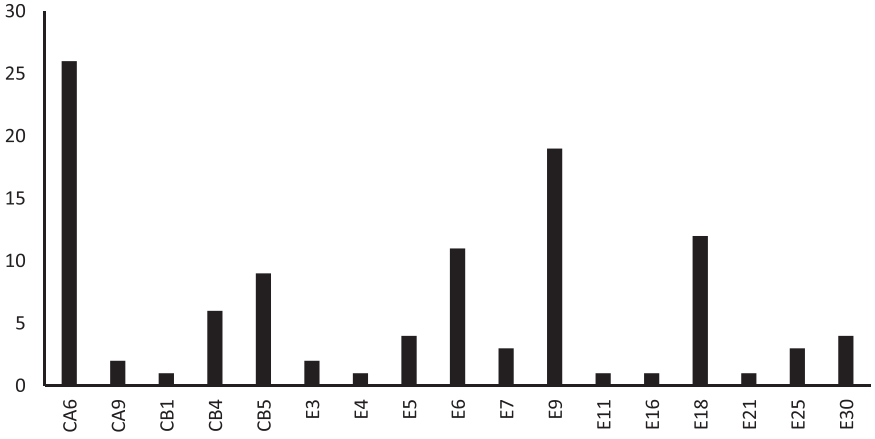

Fig. 1. Showing the distribution of EV serotypes in this UK Midlands population during a 3-year period (2014–2016, inclusive). CA–Coxsackie A; CB–Coxsackie B; E–echovirus.

or adult meningitis, were included during this 3-year retrospective study period. Clinical data was extracted for each of these patients and were compared between EV serotypes using the Fisher Exact or Mann-Whitney test as appropriate (Table 1). Enterovirus serotyping was performed at the UK national reference laboratory (Public Health England, London, Colindale, PHE Enteric Virus Unit), using partial VP1 gene sequences, as described elsewhere.[2]

Of the 102 patients with samples that had sufficient viral load for further EV typing, 27 had Coxsackie A (CAV), 15 Coxsackie B (CBV) and 60 echovirus; the most common EV serotypes detected (in decreasing order of frequency) in this mixed paediatric and adult patient population were CA6, E9, E18, E6 and CB5 (Fig. 1).

Of the 27 CAV infections, only 3 (2 CA6, 1 CA9) were found in CSF, the rest were found in skin (n = 22) or throat swabs (n = 2). So in this cohort, CAV caused mainly skin infections and nearly all of these were due to CA6. At least 7 of these CA6 infections presented with eczema herpeticum-like vesicular or more florid rashes, with the remainder presenting with more typical HFMD.

In addition, one case of EVD68 infection was found, detected in the respiratory swab of an ex-premature, 5-month old baby with bronchiolitis, discharged home after a 5-day stay. A case of EV71 infection was found, detected in a skin swab from a 2-year old child with uncomplicated HFMD, who was discharged home on the same day of admission. Neither case had any neurological complications.

As nearly all of CAV positive samples were skin swabs taken from relatively well patients with some form of skin rash or HFMD, statistical analysis was only performed for CBV versus echoviruses, where CSF was taken as part of the diagnostic workup for the more severely ill cases. The analysis for this combined CBV and echovirus cohort showed that CBV was associated with increased length of stay (LOS), higher lymphocytosis, higher CSF protein and higher CSF lymphocytosis, but lower CRP (C-reactive protein), compared to echoviruses (Table 1).

The paediatric (0–18 years) and adult (>18 years) cases were also analysed separately (Table S1). In the paediatric cohort, only the CSF protein was significantly different, with Coxsackie B virus infections resulting in higher CSF protein than echovirus infections. However, this parameter was not significantly different between Coxsackie B and echovirus infections in the adults (> 18 years). In the adult cohort, the CSF glucose and white cell counts were both significantly higher in Coxsackie B than echovirus infections.

Although the clinical management is similar (in the absence of any specific antiviral treatment) for all EV serotypes, the availability of real-time serotyping may be useful to give clinical teams an indication of which cases may develop more severe disease, and therefore need closer monitoring – especially neonates.

Such enterovirus surveillance, particularly in paediatric patients, is important to identify specific EV serotypes that can cause more severe disease, e.g. EVD68 and EV71, which are currently relatively rare in this UK population. These serotypes are well-known to be a cause of hand-foot-mouth disease, but in particular, have been frequently reported to cause more serious neurological complications, including acute flaccid paralysis and encephalomyelitis.[2]

Surveillance also allows us to detect any change in the predominant cause of HFMD. Noticeably in this cohort, nearly all the non-CSF, skin swab samples were due to CA6 in children presenting with some form of HFMD. Although historically, the majority of HFMD has been caused by CA16 and EV71, in recent years a new lineage of CA6 causing a more severe form of atypical HFMD has been reported from different countries,[3] including China,[4] Finland,[5] Japan,[6] France,[7] the USA,[8] and Denmark.[9] Several of these reports highlighted the wider range of rash presentations associated with CA6 HFMD, which were not seen in traditional CA16 or EV71 HFMD, e.g. as disseminated zoster-like presentations, peri–oral rashes and high fever with vesiculobullous skin lesions, demonstrating the ability of CA6 infections to mimic several other skin conditions, e.g. eczema herpeticum, vasculitis, syphilis, erythema multiforme and Stevens-Johnson syndrome.[6–9]

The increased LOS, higher lymphocytosis, higher CSF protein and higher CSF lymphocytosis in the CBV infections, suggests a greater host central nervous system (CNS) inflammatory response to the CBV than echovirus in this cohort (Table 1). The same trend (CBV causing more inflammation than echovirus) is seen when the paediatric and adult cohorts are analysed separately, though the combination of parameters that show a significant difference change slightly, and are all CSF-related (Table S1). This subgroup analysis indicates that CBV infections in both children and adults cause a more intense CNS infection and inflammatory response than infection with echoviruses.

In conclusion, in this mixed paediatric and adult UK Midlands population, presenting with sepsis or viral meningitis, multiple EV serotypes were identified in CSF samples. Analysis of patients' laboratory data suggested more severe disease caused by Coxsackie B viruses compared to echoviruses, though larger studies are warranted to confirm this finding. Periodic, national surveillance will alert paediatricians to the presence of different enteroviruses circulating in the community, including the emergence of any new and significant EV types (such as CA6 HFMD), and prepare them to potentially manage more cases of severe disease in some patients, including possible treatment with intravenous immunoglobulin (IVIG).[10]

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Supplementary materials

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