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Letter to the Editor

Neurosurgical device-associated infections due to *Candida auris* – Three cases from a single tertiary center

Dear Editor,

Candida auris has recently emerged as a novel global fungal pathogen causing nosocomial infections in six continents. Outbreaks have occurred in 30 countries worldwide and recent reports in this journal have highlighted the experiences of various centers in managing outbreaks and cases of fungaemia due to *Candida auris*.[1,2] Work is ongoing to elucidate the pathogenesis and clinical management of this novel pathogen.[3]

Nosocomial infections due to candida species are on the rise worldwide although neurosurgical infections are rare. In general infections associated with neurosurgical devices are associated with a significant increase in morbidity and mortality and may pose diagnostic and therapeutics challenges.[4]

We have recently described a prolonged *Candida auris* outbreak in our neurosciences intensive care unit involving a total of 70 patients between February 2, 2015, and August 31, 2017.[5] Here, we describe our clinical experience in managing three patients with central nervous system device-associated infections due to *Candida auris* during this outbreak. These were caused by isolates that were resistant to triazole antifungals, adding to the difficulties in treating these difficult cases. The demographic and clinical features of these patients are summarized in Table 1.

Case 1

A 46-year old female was admitted with an extensive basal subarachnoid haemorrhage with associated malignant hypertension. This was managed with bi-frontal craniectomy and placement of an external ventricular drain (EVD). Her medical background included hypothyroidism, diet-controlled diabetes and mild depression. She developed multiple nosocomial infections including ventilator-associated pneumonia, Methicillin-susceptible *Staphylococcus aureus* bacteraemia as well as *Enterobacter cloacae* EVD-associated ventriculitis. The latter was managed with EVD exchange and three weeks of intravenous and intraventricular antibiotics with early sterilization of cultures and subsequent placement of a ventriculo-peritoneal shunt. During rehabilitation, 16 weeks following her initial admission, she developed fever and blood cultures were positive for yeast that was subsequently identified as *Candida auris*. The peripherally inserted central catheter was removed and she received 7 days of intravenous micafungin with a rapid clinical and microbiological response with no evidence of invasive candidiasis. Four days later revision of the ventriculo-peritoneal shunt was required due to breakdown of superficial skin overlying the shunt valve. Cultures of cerebrospinal fluid (CSF) and the reservoir system were positive for *Candida auris*. Micafungin was restarted and the entire shunt system was removed and an EVD was placed. However, sterilization of the CSF cultures was only achieved on day 16 of antifungal treatment after further replacement of the EVD to the contralateral side. She received a total of 6 weeks of micafungin with no further *Candida auris*-associated complications and placement of a new ventriculo-peritoneal shunt. Twelve months after the initial event she remained in a prolonged disorder of consciousness with likely limited potential for recovery.

Case 2

A 48-year old otherwise healthy woman was admitted with a subarachnoid haemorrhage that was managed with percutaneous coil embolization of the ruptured cerebral artery aneurysm and EVD insertion. Three weeks later she developed intermittent temperatures with no obvious focus of infection. CSF cultures were positive for *Candida auris*. The EVD was exchanged 72 h after initiation of effective antifungal therapy with rapid sterilization of CSF cultures. She received a total of 15 days of systemic antifungals consisting of 1 day of fluconazole followed by 14 days liposomal amphotericin. Six weeks later a ventriculo-peritoneal shunt was inserted without any subsequent infective complications. She made a good clinical recovery and on review six months later was merely requiring rehabilitation for short term memory loss and difficulties in writing.

Case 3

A 71-year old male patient presented with seizures, meningitis and radiological evidence of bilateral frontal collections with associated ventriculitis. He had an extensive neurosurgical history that included transsphenoidal surgery for pituitary adenoma 14 years and frontal craniotomy for fibrodysplasia of the skull 6 years prior to presentation, respectively. The latter procedure had resulted in persistent communication between frontal sinus and the nasal cavity. Furthermore, he had previously received treatment for non-Hodgkins lymphoma twice with successful remission. His management included evacuation of pus from both frontal cavities, prolonged extraventricular drainage, courses of intravenous antibiotics and finally insertion of a ventriculo-peritoneal shunt eight weeks following initial presentation. A few months later he deteriorated clinically with evidence of a CSF leak and complex intracranial infection with associated pneumocephalus requiring endoscopic surgical repair of the skull base defect. The CSF leak persisted with subsequent detection of non-albicans Candida from cultures of the pneumocephalus cavity and subsequently also the CSF. Antifungal treatment with voriconazole was initiated that was subsequently changed to liposomal amphotericin and flucytosine on day 10 after culture results demonstrated growth of *Candida auris*. A number of surgical procedures including EVD placements were required until complete removal of the ventriculo-peritoneal shunt could be undertaken six weeks later. Cultures of the CSF and

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Table 1
Demographics and clinical features of three cases of neurosurgical infections due to *C. auris*.

	Case 1	Case 2	Case 3
Age, gender	46y, F	48y, F	71y, M
Admission diagnosis	Subarrachnoid haemorrhage	Subarrachnoid haemorrhage	Bi-frontal intracerebral collections and ventriculitis
Co-morbidities	Diabetes, hypothyroidism	None	Extensive neurosurgical intervention, non-Hodgkins lymphoma
Prior total courses antibiotics (total days)	10 (53)	2 (10)	3 (65)
Site of infection	Ventriculo-peritoneal shunt	EVD	Ventriculo-peritoneal shunt, intracerebral
Time in hospital to 1st positive *C. auris* CSF	17 weeks	3 weeks	13 weeks
CSF WCC ($x10^6$/L)	16 (100% lymphocytes)	20 (100% lymphocytes)	36 (83% lymphocytes)
C. auris positive sites[a]	Blood, CSF, shunt reservoirs and tissue	CSF	CSF, shunt reservoirs, cerebral tissue and fluid
Duration of positive culture	18 days	6 days	46 days
Antifungal treatment	Micafungin	Liposomal amphotericin B	Liposomal amphotericin B and flucytosine/micafungin
Total duration antifungals[b]	62 days	14 days	42 days
Surgical treatment	Shunt exchange	EVD replacement	Debridement, shunt exchange

Abbreviations: EVD – extraventricular drain, WCC – white cell count, CSF – Cerebrospinal fluid.
[a] Excludes colonization.
[b] Excludes days of non-effective azole treatment.

intraoperative tissue samples remained positive throughout this period with development of flucytosine resistance four weeks after initiation of dual treatment despite adequate monitoring of therapeutic flucytosine levels. Flucytosine was subsequently replaced with micafungin. Surgical and antimicrobial treatment remained challenging with poor outcome of overall neurological recovery. Furthermore, 19 days later, whilst CSF cultures were still intermittently positive for *C. auris*, replacement of the ventricle-peritoneal shunt was required in order to manage worsening hydrocephalus. Eventually cultures became sterile and antifungals could be ceased two weeks after shunt insertion. Three months later the patient was repatriated to his local hospital where he died the following year of non-related causes.

Discussion

Here, we present our experience in successfully treating three distinct cases of device-associated central nervous system infection due to *Candida auris* during an emerging outbreak of this novel pathogen in our unit.

All three cases displayed risk factors for invasive candidiasis including antimicrobial exposure, prolonged ICU stay and presence of medical devices. Echinocandins are considered first line agents for invasive infections due to *Candida auris* but have low penetration into central nervous system.[3,6] Interestingly, two of our cases received micafungin, one as sole agent and the other to replace flucytosine as adjunctive treatment to liposomal amphotericin, with successful microbiological cure. Neither of our cases required administration of intraventricular antifungals, although use of intraventricular caspofungin in a case of recalcitrant *Candida auris* ventriculo-peritoneal shunt infection has recently been described.[7]

Our experience demonstrates that key to successful management of device-associated *Candida auris* infections is a multidisciplinary approach with timely source control and removal of prosthetic devices in addition to prolonged administration of targeted antifungal therapy.

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Elham Khatamzas*
NHS Lothian Edinburgh, United Kingdom

Hilary Madder, Katie Jeffery
Oxford University Hospitals NHS Foundation Trust, United Kingdom

*Corresponding author.
E-mail address: ekhatamzas@doctors.org.uk (E. Khatamzas)

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Subtherapeutic posaconazole troughs despite high-dose posaconazole tablets in a patient with terminal ileum resection

Dear Editor,

We read with interest a recently published article by Epstein et al., where the use of intravenous micafungin as an alternative antifungal prophylaxis to oral posaconazole was suggested in patients undergoing chemotherapy for acute leukemia and myelodysplastic syndrome.[1] In this study, we note that only 54% of patients prescribed with posaconazole suspension achieved therapeutic posaconazole level of >0.7 mg/L, the recommended level for anti-fungal prophylaxis.[1,2] This further highlights the constraints of achieving adequate levels with posaconazole suspension, consistent with its known unpredictable and poor bioavailability.[2,3] On the contrary, posaconazole delayed-release tablet, the new posaconazole formulation, has shown to achieve higher posaconazole levels due to its enhanced bioavailability.[3] Although posaconazole tablet is the preferred formulation to be used clinically, subtherapeutic troughs are more likely to occur among patients prescribed with standard dosing of delayed-release tablets (300 mg/day) if they weigh >90 kg, had diarrhea or were prescribed proton-pump inhibitors.[4,5] A case report also suggested that hypoalbuminemia and hyperbilirubinemia resulted in subtherapeutic troughs due to enhanced posaconazole clearance.[6] However, no report has described the use of posaconazole delayed-release tablets in patients with short small bowel. This case report describes the first case of persistent subtherapeutic posaconazole troughs despite the use of high-dose posaconazole tablets, up to 600 mg/day, in a patient who had undergone terminal ileum resection.

The patient was a 43-year-old female, weighing 40 kg, with acute myeloid leukaemia (AML). Her chemotherapy was complicated by neutropenic enterocolitis and terminal ileum perforation, for which she underwent right hemicolectomy with double barrel stoma and had 40 cm of her terminal ileum resected. She also developed a left lung apical fungal pneumonia on Computed Tomography (CT) thorax. Her serum and bronchial lavage galactomannan were not yielding. She was treated with IV liposomal amphotericin B and serial CT thorax demonstrated improvement. After 3 months, posaconazole 200 mg suspension four times a day was commenced with the aim to discontinue amphotericin B once therapeutic posaconazole trough of ≥1.0 mg/L was attained. The posaconazole trough of >1.0 mg/L has been recommended by the British Society for Medical Mycology for the treatment of invasive fungal infections.[2]

At our institution, posaconazole troughs are obtained <1 h before the next dose (≥7 days upon drug initiation). The serum was assayed using a validated high performance liquid chromatography (detection range: 0.5–7.5 mg/L). For this patient, posaconazole trough on day 7 (D7) and D13 of posaconazole suspension were <0.5 mg/L. The subtherapeutic troughs were attributed to concurrent omeprazole (20 mg once daily), which is known to reduce posaconazole absorption.[2,3] Therefore, posaconazole dose was increased multiple times over the next 3 weeks, with each dose administered with peanut butter (Table 1). The trough was therapeutic at 1.75 mg/L on D34 with posaconazole 400 mg four times a day and amphotericin B was stopped. A repeat trough on D41 was 2.4 mg/L and no posaconazole-associated side effects were observed.

Subsequently, posaconazole tablet was introduced into the hospital formulary and posaconazole suspension was switched to tablet 300 mg (a cheaper alternative) once daily with food on D42. Omeprazole was discontinued. On D48, the trough was <0.5 mg/L. Hence, a loading dose of 300 mg twice daily was administered for one day, followed by 300 mg once daily. However, trough on D54 remained <0.5 mg/L. Posaconazole dose was subsequently increased to 200 mg twice daily and 200 mg thrice daily but troughs were <0.5 mg/L (D69) and 0.57 mg/L (D75), respectively. Due to logistical limitations and delay in posaconazole trough reporting, the posaconazole therapy was only modified on D91. In view of persistent sub-therapeutic troughs with posaconazole tablet, posaconazole suspension 400 mg four times a day with peanut butter was restarted without acid-reducing agents on D91. Posaconazole trough was 4.1 mg/L on D110 and no posaconazole-related adverse events were noted. Posaconazole dose was decreased to 300 mg four times a day to reduce cost. The troughs were 1.9 mg/L and 1.5 mg/L on D138 and D161, respectively. She passed away three months later due to AML.

During the receipt of posaconazole tablets, administration with food was ensured as per manufacturer recommendation. There was strict medication compliance and no drug–drug interactions were identified. No vomiting or diarrhoea (consistent stoma output) was observed. Moreover, there were neither severe hypoalbuminemia (albumin 31–42 g/L) nor hyperbilirubinemia (bilirubin 11–15 umol/L) that could result in enhanced drug clearance. Therefore, we hypothesize that the shortened small intestine could have led to sub-optimal posaconazole absorption. It has been demonstrated that the distal small intestine is known to play a more important role in posaconazole absorption compared to proximal regions under the fed state.[7] This postulation was supported by the fact that compared to the fasted state, the fed state resulted in (1) less dissolution of posaconazole tablet in the jejunum; (2) higher systemic posaconazole exposure and (3) longer time required to reach peak plasma concentration compared to fasted state. Possibly, tablet administration on a fasted state or dose increase beyond 600 mg/day may result in higher troughs. However, dose increase is costly and exposing the patient to prolonged subtherapeutic levels while elucidating the optimal dose should be avoided.

Conversely, posaconazole suspension achieves dissolution in the stomach, followed by absorption in the upper small intestine.[8] Thus, reduction in posaconazole suspension dose from 1200 mg/day to 800 mg/day (standard dose) may result in therapeutic trough in the absence of acid-reducing agents in our patient. Unfortunately, this could not be ascertained.

In conclusion, posaconazole delayed-release tablets should be used with extreme caution in patients with terminal ileum resection. The use of posaconazole suspension along with therapeutic drug monitoring should be considered as the first choice therapy instead.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Table 1
Timeline of posaconazole dosing regimen and corresponding posaconazole troughs PO, by mouth.

Day	Posaconazole Formulation	Posaconazole Dose	Day	Posaconazole trough (mg/L)	Remarks
1	Suspension	200mg 4 times daily			PO omeprazole
			7	<0.5	PO omeprazole
			13	<0.5	PO omeprazole
15	Suspension	400mg (morning), 200mg (noon), 400mg (night)			PO omeprazole
			21	<0.5	PO omeprazole
22	Suspension	400mg 3 times daily			PO omeprazole
			27	<0.5	PO omeprazole
28	Suspension	400mg 4 times daily	34	1.75	PO omeprazole
			41	2.4	PO omeprazole
42	Delayed-release tablet	300mg once daily	48	<0.5	
49	Delayed-release tablet	300mg twice a day for 1 day, then 300mg once daily	54	<0.5	
55	Delayed-release tablet	200mg 2 times daily	69	<0.5	
70	Delayed-release tablet	200mg 3 times daily	75	0.57	
91	Suspension	400mg 4 times daily	110	4.1	
111	Suspension	300mg 4 times daily	138	1.9	
			161	1.5	

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Peijun Yvonne Zhou, Tze Peng Lim, Si Lin Sarah Tang, Hui Ling Winnie Lee
Department of Pharmacy, Singapore General Hospital, Outram Road, Singapore 169608, Singapore

Thuan Tong Tan, Ban Hock Tan
Department of Infectious Diseases, Singapore General Hospital, Outram Road, Singapore 169608, Singapore

Si Yun Melinda Tan, Gee Chuan Wong
Department of Haematology, Singapore General Hospital, Outram Road, Singapore 169608, Singapore

Lay Hoon Andrea Kwa*
Department of Pharmacy, Singapore General Hospital, Outram Road, Singapore 169608, Singapore
Emerging Infectious Diseases, Duke-NUS Medical School, Singapore, Singapore
Singhealth Duke-NUS Medicine Academic Clinical Programme, Singapore

*Corresponding author at: Department of Pharmacy, Singapore General Hospital, Outram Road, Singapore 169608, Singapore.
E-mail address: andrea.kwa.l.h@sgh.com.sg (L.H.A. Kwa)

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Use of enteral amikacin to eliminate carriership with multidrug resistant Enterobacteriaceae

Dear Editor,

We read with interest the article by Prim and co-workers concerning colistin resistant Enterobacteriaceae in hospitalized patients.[1] Critically ill patients with prolonged hospitalization, are at increased risk of acquiring infections with Enterobacteriaceae. The routes of infection are either endogenous, following colonization of the gastro-intestinal tract, or exogenous when hygiene fails.[2] Selective digestive tract decontamination (SDD) is designed to prevent secondary endogenous infection by eliminating abnormal carriership with potential pathogenic microorganisms (PPM) with topical antimicrobial agents.[3] The commonly used topically applied antimicrobial substances in SDD are polymyxin, tobramycin and amphotericin B (PTA). Eradication of carriership with PPM's is usually effective with these agents but may be difficult in case of multidrug resistant gram-negative bacteria (MDRGNB).[4,5] This is particularly true in case of combined resistance to colistin and tobramycin. PPM's resistant to these antimicrobial agents need other substances to achieve successful eradication.[6]

We report three cases of colonisation with MDRGNB that were treated with topical amikacin.

Case 1: *Enterobacter cloacae*

A male tourist from Surinam, 68 years old, was admitted to our ICU with loss of consciousness due uraemia caused by acute kidney injury as the result of a *de novo* HIV infection with a first viral load count of 2.1 10^6 copies/ml. He had no relevant medical history, but in the previous months his physical condition deteriorated. He was emaciated due to lack of self-care and understanding of his disease. He had developed a producing cough and distinct coccygeal decubitus.

On admission to the ICU, renal replacement therapy and antiretroviral therapy was started with dolutegravir and emtricitabine/tenofovir. SDD was given with PTA 2% in an oral paste (Orabase®) and in a suspension containing 100 mg of polymyxin B sulphate, 80 mg of tobramycin sulphate and 500 mg amphotericin B four times daily in the nasogastric tube. Cefotaxime 1 g was given

Table 1
(a)–(c): Growth density (no. of colonies/ml) of MDRGNB in surveillance cultures during ICU stay; day 0 indicates the start of topical amikacin.

(a)								
Rectum	>1000	1000	<15	0	0	0	0	
Throat	0	<15	0	0	0	0	0	
Sputum	0	0	0	0	0	0	0	
Day	−5	−1	+2	+6	+9	+13	+16	
(b)								
Rectum	100–1000	15–100	15–100	>1000	100–1000	<15	0	0
Throat	0	0	0	0	0	0	0	0
Sputum	0	0	0	0	0	0	0	0
Day	−7	−4	−2	+1	+5	+12	+15	+18
(c)								
Rectum	100	15–100	0	100	0	100	0	
Throat	0	0	0	0	0	0	0	
Day	−1	+3	+6	+10	+13	+17	+20	

4 times daily i.v. and ciprofloxacin 400 mg 2 times daily i.v. for 4 days until cultures of trachea, blood and urine appeared without growth. Surveillance cultures of throat, tracheal aspirate and rectum were taken on admission and twice weekly and showed multidrug resistant *Enterobacter cloacae* as shown in Table 1. This Extended Spectrum Beta-lactamase (ESBL) producing *E. cloacae* was resistant for all cephalosporins, gentamicin, tobramycin, fluoroquinolones and polymyxins but susceptible for amikacin and meropenem detected by agar disk-diffusion following the EUCAST guidelines.

To eliminate this abnormal carriership, amikacin was added to the SDD regimen, the start is indicated as day 0 in Table 1(a). Amikacin 4 times daily 500 mg of the i.v. fluid was given through the nasogastric tube and 4 times daily 500 mg of the i.v. fluid was applied with a disposable oral swab throughout the complete oral cavity from day 0 till end of ICU admission. Aerosolized amikacin was given 500 mg 4 times daily from day 6–13[5]. Persistent elimination of carriership of this *E. cloacae* over time was observed. This patient died because of refractory multiple organ failure at day 16 despite intensive treatment.

Case 2: *Proteus mirabilis*

An 81-years old woman was repatriated from a visit to Turkey were the Guillain–Barre syndrome was diagnosed. She was mechanically ventilated because of muscle weakness. The rectal surveillance cultures showed several Enterobacteriaceae including a multidrug resistant *Proteus mirabilis*. This MDRGNB was sensitive to amikacin but not to any other tested antimicrobial drugs, including tobramycin and colistin. On admission standard SDD with PTA was started as described in case 1. A week after admission amikacin orally and enterally was started in the same way as in case 1. Table 1(b) shows the decrease in colony forming units (CFU) per ml faeces. Day 0 indicates the start of topical amikacin treatment.

Case 3: *Morganella morganii*

A 57-years old woman was admitted to the normal hospital ward because of acute myeloid leukaemia. She was treated with intensive chemotherapy. During this course oral ciprofloxacin 500 mg twice daily and oral fluconazole 200 mg once daily was prescribed as prophylaxis. She did not use SDD as described in cases 1 and 2 and was not admitted to the ICU. Persistent abnormal carriership with *Morganella morganii*, resistant for ciprofloxacin, tobramycin and colistin, was treated with oral amikacin 4 times daily 500 mg. Amikacin was not distributed with an oral swap as in aforementioned cases, because the patient had normal oral intake. Table 1(c) shows the results of surveillance cultures over time. In this last case it took longer to obtain eradication.

Enteral amikacin for abnormal colonization with Enterobacteriaceae in the digestive tract of patients can be successful. Case 3 shows that in the non-ICU patient with normal oral intake this strategy might be less successful. Further studies are needed to assert its efficacy.

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Declarations of interest

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Jennilee Nahar, Sophie Buitinck
Department of Intensive Care Medicine, OLVG Hospital, P.O. Box 95500, 1090HM Amsterdam, the Netherlands

Rogier Jansen
Department of Medical Microbiology, OLVG Hospital, P.O. Box 95500, 1090HM Amsterdam, the Netherlands

Eric A.F. Haak
Department of Clinical Pharmacy, OLVG Hospital, P.O. Box 95500, 1090HM Amsterdam, the Netherlands

Peter H.J. van der Voort*
Department of Intensive Care Medicine, OLVG Hospital, P.O. Box 95500, 1090HM Amsterdam, the Netherlands
TIAS School for Business and Society, Tilburg University, Warandelaan 2, 5037AB, Tilburg, the Netherlands

*Corresponding author at: Department of Intensive Care Medicine, OLVG Hospital, P.O. Box 95500, 1090HM Amsterdam, the Netherlands.
E-mail address: p.h.j.vandervoort@olvg.nl (P.H.J. van der Voort)

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A novel machine-learning-based infection screening system via 2013–2017 seasonal influenza patients' vital signs as training datasets

Dear Editor,

We previously reported in *Journal of Infection* an infection screening method using a combination of neural network and k-means clustering algorithm.[1] After a pandemic of a novel influenza A/H1N1pdm09 in 2009, we started to develop vital sign-based infection screening systems and conducted several preliminary infection screenings in Japan, Mongolia and Vietnam.[2–4] Although the previous method achieved up to 98% sensitivity and 96% negative predictive value (NPV), this study was limited to patients with comparatively uniform backgrounds, i.e., hospitalized influenza patients who were soldiers of the Japan Self-Defense Forces. To apply this system in patients with a variety of clinical backgrounds, we newly developed a machine learning-based infection screening system that incorporates a random tree algorithm (RTA) using system-derived vital signs as training datasets, i.e., heart rates (HR), respiratory rates (RR) and estimated axillary temperatures (T_{EAX}) of 189 seasonal influenza patients (6–92 years) acquired over the 4 influenza seasons from 2013 to 2017 in Japan at a family doctor's office and those of 189 healthy volunteers (18–85 years). Using the proposed system, we conducted influenza screening within 20 s for 52 patients (8–92 years) and 52 healthy volunteers (21–60 years) in the 2017–2018 influenza cases. Influenza virus isolation was performed using the Madin–Darby canine kidney (MDCK) cell system. The patients were recruited at Takasaka Clinic, Fukushima, Japan. Healthy volunteers were students and staffs of Tokyo Metropolitan University and The Institute of Medical Radiology Technologists at the Japan Self-Defense Force Central Hospital. The present study was approved by the Ethics Committees of Tokyo Metropolitan University, Hino Campus.

In this study we adopted a modified version of the infection screening system[5] that was newly equipped with a temperature calibration function for accurate axillary temperature estimation via non-contact derived facial temperature (T_F) (Fig. 1(a)). Estimated axillary temperatures (T_{EAX}) was calculated using the following equation:

$$T_{EAX} = 0.55T_F - 0.82T_A + 35.8 \quad (1)$$

Where T_F is the facial skin temperature measured by the infrared thermography camera, T_A is the ambient temperature determined by a built-in thermistor.

We used the WEKA 3.8.1 machine-learning software for RTA determination via training datasets. RTA can be expressed using a flow-chart, and it can be easily converted using final software production rules, because it can be expressed as "if–then" statements by the software.[6–8]

In influenza virus isolation, oropharyngeal and nasopharyngeal swabs were collected from the patients as the clinical specimens and they were inoculated onto MDCK followed by incubation in the 5% CO_2 incubator at 34 °C for one week at the Virus Research Center, Sendai Medical Center, National Hospital Organization, Sendai, Miyagi, Japan. Influenza viruses type A, H1N1, and H3N2 subtypes, type B, Victoria and Yamagata lineages, and type C were isolated.

Fig. 1(b) shows RTA-based classification trees respectively corresponding to 2013–2014 (46 influenza patients and 46 healthy volunteers), 2013–2014 and 2014–2015 (92 influenza patients and 92 healthy volunteers), 2013–2014, 2014–2015, and 2015–2016 (144 influenza patients and 144 healthy volunteers), and 2013–2014, 2014–2015, 2015–2016, and 2016–2017 (189 influenza patients and 189 healthy volunteers). Mean HR, RR, and T_{EAX} of the patients in 2016–2017 were higher than those of the patients in 2013–2014. In 2016–2017, 83% of the patients were infected with A(H3N2), where 59% of the patients were infected with B/Victoria in 2013–2014. Previous research suggests that A(H3N2) was associated with more severe symptoms than B/Victoria.[9,10]

Sensitivity and NPV were determined for the 2017–2018 test set including 52 influenza patients and 52 healthy volunteers using the RTA-based classification trees incorporating data sequentially accumulated from the influenza seasons from 2013 to 2017. Sensitivity and NPV increased as data from more previous influenza seasons were incorporated into the RTA-based classification tree (Fig. 2(a)). Fig. 2(b) shows the infection screening results (as a function of axillary temperatures determined by a contact type thermometer) of the 2017–2018 test set using the RTA-based classification tree derived using data from all influenza seasons from 2013 to 2017 as the training dataset.

Although 40% of the influenza patients being nonfebrile, the RTA-based infection screening system achieved 96.2% sensitivity and 96.0% NPV for the patients with a variety of clinical backgrounds, while our previous method using the combination of a neural network and k-means exhibited 84.6% sensitivity and 84.3% NPV using the same test datasets.[1] Also, the sensitivity of our proposed method using the training dataset incorporating four influenza seasons was 96.2%, more than 15% higher than that using dataset from just one influenza season (2013–2014). This can be attributed to the fact that the 2013–2014 training dataset does not include data from patients infected with B/Yamagata, which was the dominant lineage in the 2017–2018 test set. The location where we collected our data, Takasaka Clinic, is near Yamagata where B/Yamagata lineage was firstly isolated. The sensitivity and the NPV of the proposed system increased each year, and these increases are attributed to increases in annual training dataset numbers and corresponding RTA-based classification tree growth.

Regarding implementation, the RTA-based infection screening system is easily convertible to final production as software, because the algorithm consists of simple combinations of "choice between the two" operations. This simplicity of the RTA-based infection screening system facilitates a computation time that is 600 times faster (0.02 s, excluding the time taken to measure vital signs) than that of the previous method.

The present study might have a limitation. While the study did include patients with various clinical backgrounds, all the patients were recruited from a single facility, the Takasaka Clinic in Fukushima, Japan. Influenza symptom severity and human vital signs may differ slightly by global geographical location. Thus, there is a need to verify the performance of RTA in clinical trials conducted in countries with tropical, subtropical and temperate climates.

In conclusion, RTA-based infection screening systems using training datasets appear promising for future rapid and accurate screening for influenza in patients with a variety of clinical backgrounds.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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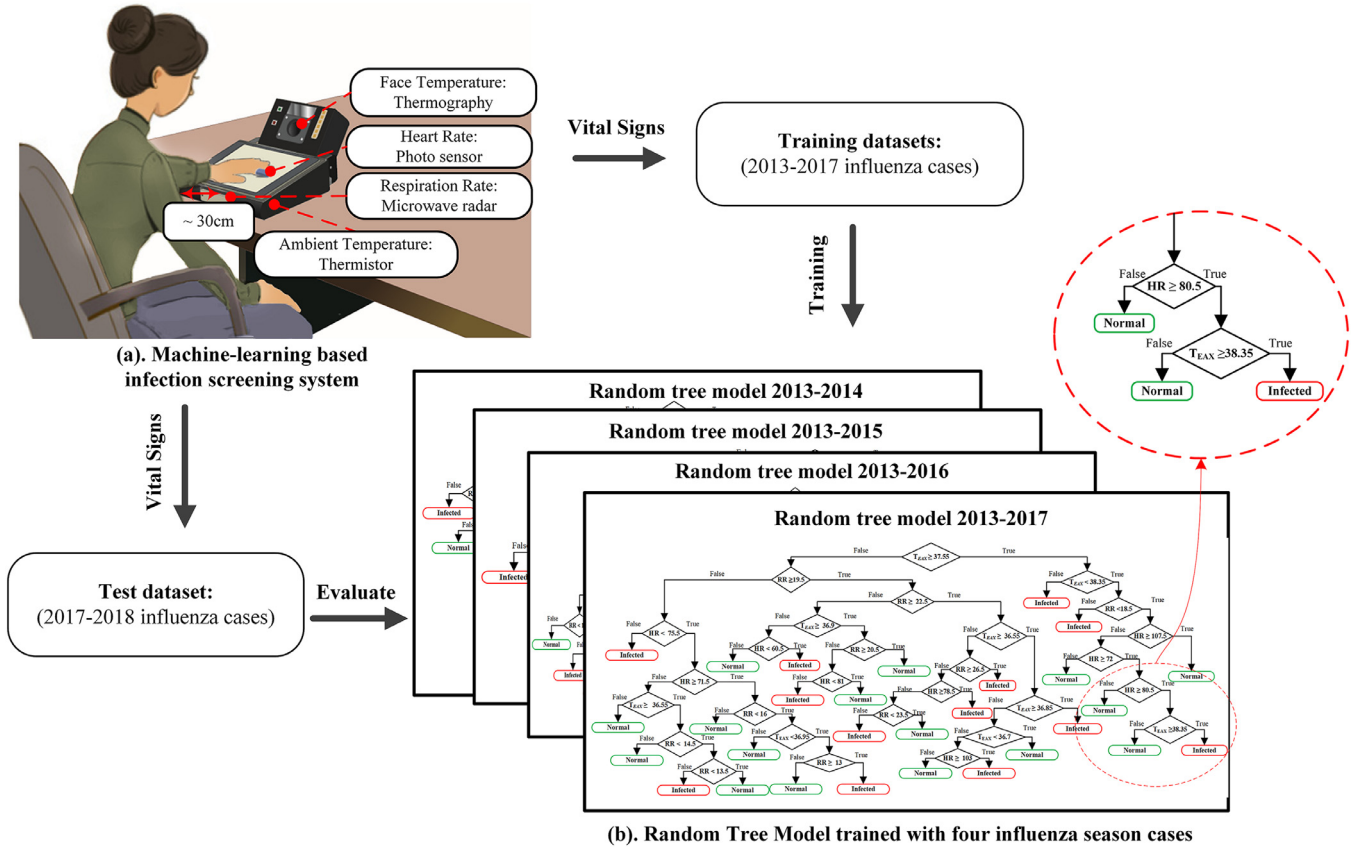

Fig. 1. Schematic diagram of the measurement and evaluation procedure. (a) Machine-learning based infection screening system (b) random tree models trained with consecutive four years influenza cases.

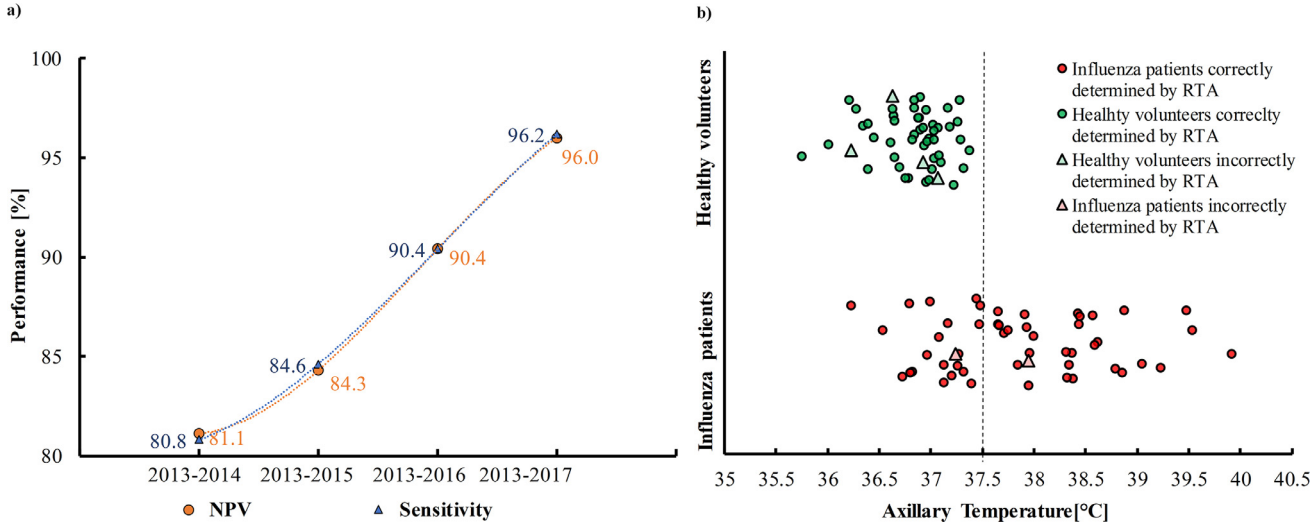

Fig. 2. Screening results derived from the 2017–2018 test set (52 influenza patients and 52 healthy volunteers). (a) Sensitivity values and negative predictive values (NPVs) were determined using the cumulative total amount of data available after each influenza season from 2013 to 2017. (b) Screening results using a random tree algorithm (RTA)-based classification tree generated using data from all influenza seasons from 2013 to 2017 (189 influenza patients and 189 healthy volunteers) as a function of axillary temperature determined by a contact type thermometer.

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Sumiyakhand Dagdanpurev*
Graduate School of Systems Design, Tokyo Metropolitan University, Tokyo 191-0065, Japan
Machine Intelligence Laboratory, National University of Mongolia, Ulaanbaatar 14201, Mongolia

Shigeto Abe
Takasaka Clinic, Iwaki, Fukushima 973-8407, Japan

Guanghao Sun
Graduate school of Informatics and Engineering, University of Electro-Communications, Tokyo 182-8585, Japan

Hidekazu Nishimura
Virus Research Center, Clinical Research Division, Sendai Medical Center, National Hospital Organization, Sendai 983-8520, Japan

Lodoiravsal Choimaa
Machine Intelligence Laboratory, National University of Mongolia, Ulaanbaatar 14201, Mongolia

Yukiya Hakozaki
Genkikai Yokohama Hospital, Yokohama 226-0013, Japan

Takemi Matsui
Graduate School of Systems Design, Tokyo Metropolitan University, Tokyo 191-0065, Japan

*Corresponding author.
E-mail address: sumiyakhand-dagdanpurev@ed.tmu.ac.jp
(S. Dagdanpurev)

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Next-generation sequencing combined with routine methods to detect the pathogens of encephalitis/meningitis from a Chinese tertiary pediatric neurology center

Dear Editor,

In this paper, 153 children were studied and the results supported a review published in this journal on the next-generation sequencing of pathogens.[1] NGS can be used as a new method for the detection of pathogens, especially for unexplained encephalitis/meningitis. Encephalitis is a serious central nervous system (CNS) inflammatory disease involving the brain parenchyma. Meningitis is a disease involving the meninges, inducing headache, increased intracranial pressure, and severe brain involvement. Despite significant advances in the diagnosis and treatment of encephalitis/meningitis, many cases still have unsatisfactory outcomes. In the past few decades, more than half of infected children in the California Encephalitis Project did not receive a pathogenic diagnosis. Among 1570 children with encephalitis enrolled, nearly 63% of encephalitis patients had unidentified pathogens.[2]

Many pathogens can cause encephalitis/meningitis, however, the pathogeny of up to 50% of cases worldwide is unknown.[3,4] In recent years, high-throughput DNA sequencing (HTS), also known as next-generation sequencing (NGS), has gradually gained attention because of its wide coverage in pathogen detection.[5–8] Current reports incorporating NGS are mainly case reports, and no studies on the positive rate of pathogens were published. This study will combine NGS with routine methods to detect the pathogens of cerebrospinal fluid in children with encephalitis/meningitis, and will analyze the distribution of these pathogens.

We recruited patients from February 2017 to August 2018 from different regions of China; all were inpatients of the Department of Neurology, Children's Hospital affiliated with the Capital Institute of Pediatrics. The diagnostic criteria for encephalitis/meningitis refer to the consensus on the definition of encephalitis in 2013[9] and the diagnostic criteria for encephalitis in the Beijing encephalitis group.

Cerebrospinal fluid NGS was carried out by the Binhai Genomics Institute.

In our study, there were 153 children with encephalitis/ meningitis who met the enrollment criteria, 88 males and 65 females, with an average age of 5.45 ± 3.8 years (0.17–15 years). In 90 cases clear pathogens were detected, and the most pathogen is enterovirus. (1) Routine methods: of 153 cases with cerebrospinal fluid bacterial culture, the number of positive cases was 9, accounting for 5.88%. Of 138 cases analyzed by *Mycoplasma pneumoniae* RNA-constant amplification technology detection, the number of positive cases was 23, accounting for 16.67%. Of 153 cases, the number of cases of enterovirus detected by real-time PCR was 42, accounting for 27.45%. (2) Cerebrospinal fluid NGS: Of 109 cases with NGS, 27 cases were positive, accounting for 24.77%. Routine methods combined with NGS were used in 95 children, resulting in a total of 60 positive cases. The total positive rate was 63.16%, of which the NGS positive rate was 24.77% and the routine method positive rate was 49.02%, as shown in Fig. 1. (3) The pathogens detected by the routine methods were: enterovirus, *Mycoplasma pneumoniae*, herpes simplex virus type 1, *Candida, Streptococcus pneumoniae, Enterococcus faecium, Staphylococcus aureus,* cytomegalovirus, and Epstein-Barr virus, a total of 9 types of pathogens. The number of positive pathogens detected by NGS were 27 (positive rate 24.77%, 27/109). The pathogens detected by NGS were: *Varicella zoster* virus, human herpesvirus 6A, human herpesvirus 6B, human herpesvirus type 7, herpes simplex virus type 1, adenovirus, EB virus, cytomegalovirus, parvovirus B19, *Listeria monocytogenes, Mycobacterium tuberculosis, Streptococcus pneumoniae, Haemophilus influenzae, Enterococcus faecium, Staphylococcus aureus,* and *Candida*, a total of 16 types of pathogens. The Next-generation sequencing detected pathogens but not detected by routine methods included: *Listeria monocytogenes, Haemophilus influenzae, Mycobacterium tuberculosis, Varicella zoster* virus, herpes simplex virus type 6 (Type A and Type B), herpes simplex virus type 7, adenovirus, and parvovirus B19 type. Next-generation sequencing of the two pathogens is shown in Fig. 2.

This is the first prospective study in China to use NGS combined with routine methods to detect pathogens in children with encephalitis/meningitis in a relative large sample. Based on the rou-

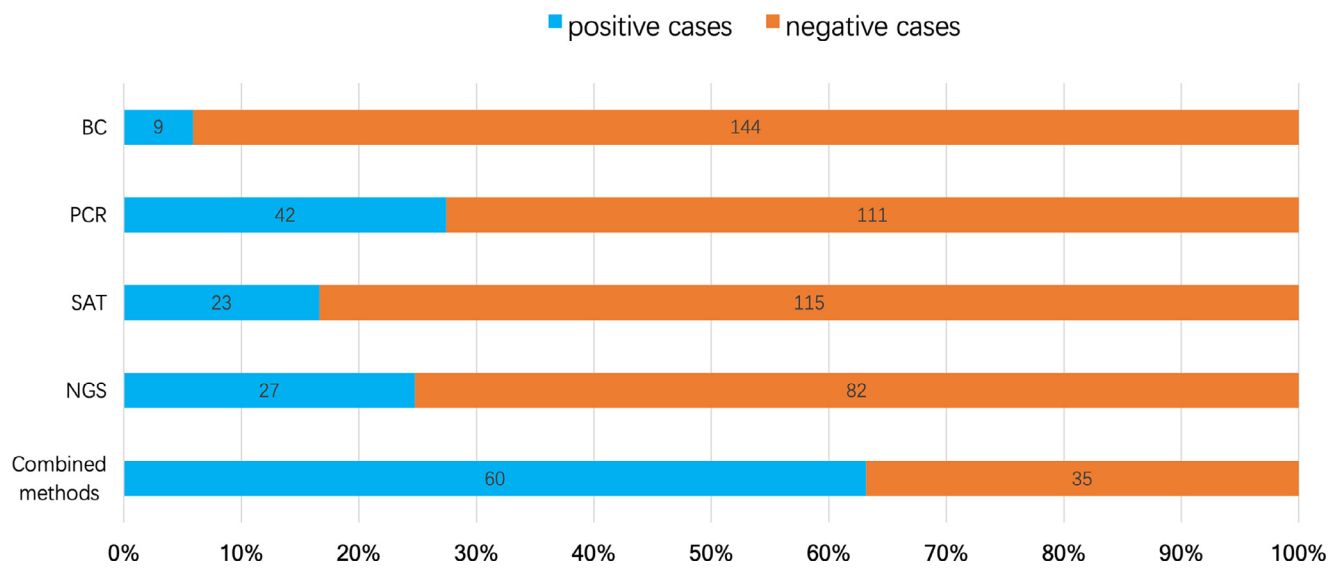

Fig. 1. NGS (next-generation sequencing), SAT (simultaneous amplification and testing), BC(bacterial culture).

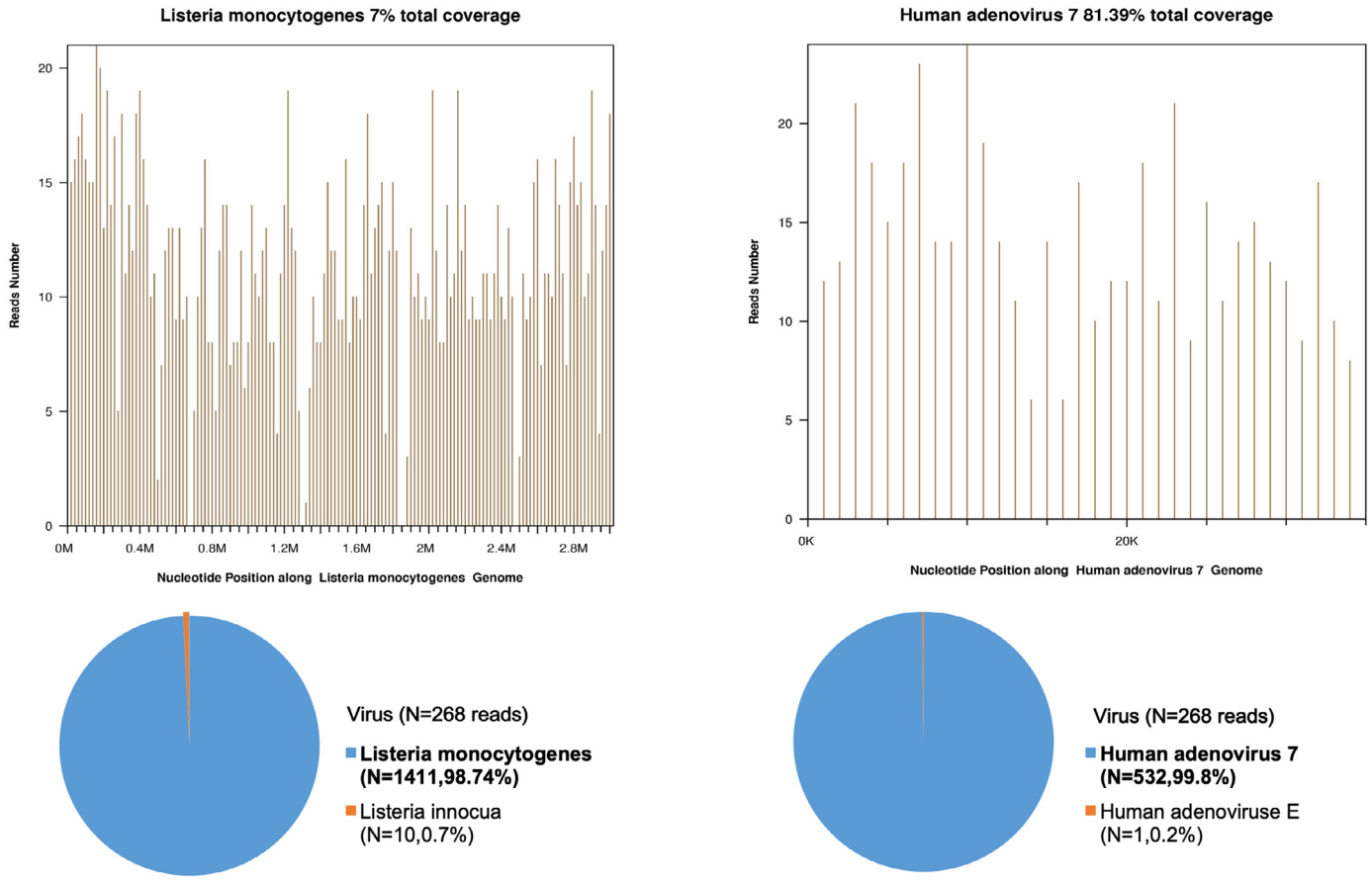

Fig. 2. Next-generation sequencing of listeria monocytogenes and adenovirus.

tine methods, NGS was used to detect pathogens in children with encephalitis/meningitis. Our research showed that routine methods combined with NGS increased the positive detection rate, and the known-pathogen spectrum was expanded.

NGS has a wide coverage of pathogens and can detect thousands of pathogens at the same time. In this study, *Listeria monocytogenes, Mycobacterium tuberculosis*, adenovirus, herpes simplex virus type 6, and human parvovirus B19 were all detected by NGS, but not by routine methods. Among these, *Listeria monocytogenes* and *Mycobacterium tuberculosis* are intracellular bacteria, and the positive rate of clinical bacterial culture is not high. NGS can separate pathogens in the cell by breaking the cell wall, thereby allowing detection of nucleic acid fragments and improving the positive rate. The overall pathogen positive rate in this study was higher than rates of most pathogens previously reported, and more types of pathogens were detected than by routine methods alone. (In

view of the low incidence of some pathogens, the routine detection of viruses in this study does not include some RNA viruses, such as Japanese encephalitis virus, West Nile virus, rubella virus, and dengue virus. If these were detected, the pathogen positive rate may have been higher.) Compared with routine methods, NGS has detected more than seven additional pathogens, providing evidence for clinical precision treatment.

Compared with routine methods, the positive rate of pathogens detected by NGS was not higher. One possible reason is that the routine methods are mainly for known pathogens, which account for the majority of infections in children with encephalitis/meningitis. The most common known pathogen of encephalitis is enteric viruses, and the enterovirus is an RNA virus. The NGS method in this study was mainly for detection of DNA viruses.

The study found that the positive rate of NGS is lower than the rutine methods, but the number of pathogens detected is more than the routine methods, so the NGS is more suitable for the pathogenic diagnosis of unexplained encephalitis/meningitis. To achieve full pathogen detection of CNS infection, routine methods are needed in combination with NGS.

Ethics statement

Ethics approval for this study was obtained from the ethics committee of the Capital Institute of Pediatrics, Beijing, China. The parents of the patients signed written informed consents and agreed that they themselves and their children would participate in this study and allow the use of relevant data and information for scientific research.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Jian-zhao Zhang, Ping Zheng
Department of Neurology, Children's Hospital Affiliated Capital Institute of Pediatrics, Beijing 100020, China

Hong-mei Sun
Department of Bacteriology, Capital Institute of Pediatrics, Beijing 100020, China

Jing-jing Dong
Department of Neurology, Children's Hospital Affiliated Capital Institute of Pediatrics, Beijing 100020, China

Shao-li Li
Department of Bacteriology, Capital Institute of Pediatrics, Beijing 100020, China

Si-yuan Fan, Hai-tao Ren
Department of Neurology, Peking Uinon Medical College Hospital of Chinese Academy of Medical Science, Beijing 100730, China

Hong-long Wu
Binhai Genomics Institute, Tianjin Translational Genomics Center, BGI-Tianjin, BGI-Shenzhen, Tianjin 300308, China

Hong-zhi Guan
Department of Neurology, Peking Uinon Medical College Hospital of Chinese Academy of Medical Science, Beijing 100730, China

Qian Chen*
Department of Neurology, Children's Hospital Affiliated Capital Institute of Pediatrics, Beijing 100020, China

*Corresponding author.
E-mail address: 13269291906@163.com (Q. Chen)

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Failure of metagenomics in detecting emerging pathogens, the *Clostridium difficile* paradigm

Dear Editor,

We read with interest in this journal, the article from Guo et al.[1] According to the authors metagenomics may hold the ability in rapid identification of different sources of abscess pathogens. We agree with the purpose but we would like to nuance metagenomic contribution in pathogens diagnosis according to the type of sample studied and especially in the case of digestive infections.

A model to highlight the respective role of culture and metagenomics for detection of digestive pathogens could be *Clostridium difficile*. Before 1978 and John Bartlett's works, there was no identified cause for pseudomembranous colitis. John Bartlett demonstrated that a bacterial toxin was responsible for the disease.[2] A cellular-free preparation obtained from the filtered caecal content of a hamster suffering from pseudomembranous colitis was inoculated in the caecum of a healthy hamster and caused

colitis. John Bartlett isolated bacteria belonging to the *Clostridia* genera from stool samples of hamsters suffering from pseudomembranous colitis, and demonstrated that these *Clostridia spp.* were responsible for the cytopathogenic effect and toxin production.[2] The *Clostridia spp.* studied belonged to the species *C. difficile*.[2] Indeed, only the pure culture of *C. difficile* has been able to highlight the pathogenicity of this bacterium in pseudomembranous colitis.

In order to observe if it was possible to diagnose *C. difficile* infection using the metagenomic tool, we tested 30 stool samples positive for *C. difficile* B toxin by real-time PCR (Xpert® *C. difficile* test by Cepheid, targeting tcdB, cdt and tcdC deletion nt 117[3]), obtained from patients hospitalized for *C. difficile* colitis, as well as 19 controls (stool samples with negative *C. difficile* real-time PCR). We performed a MiSeq® sequencing as previously described.[4] For the 30 *C. difficile* positive samples, we obtained a mean of 108,924 reads per sample. Overall, these sequences were assigned to 523 different OTUs with taxonomic classification until species level. Only 22/30 samples (73.3%) contained OTU assigned to *C. difficile*. In a second step, we performed, using Basic Local Alignment Search Tool (BLAST), a specific research for *C. difficile* sequences among the metagenomic data. We detected this sequence in five supplementary samples, i.e. 27/30 samples (90%). Indeed, without optimized analysis, metagenomics misses almost 30% of the pathogens causing the disease. This demonstrates as previously described for *C. butyricum* in necrotizing enterocolitis[5] that by metagenomics we find only what we are looking for. In parallel, in the control group, no OTU corresponding to *C. difficile* was detected by the analysis software or by manual BLAST.

Our results can be compared to those of Zhou et al., with the same approach, the authors analysed 22 *C. difficile* positive stool samples by metagenomics; compare to qPCR, *C. difficile* was detected in 90.9% of cases by 16S RNA-focused metagenomics.[6] Non-detection of *C. difficile* by metagenomics did not correlated with low abundance of the bacterium.

Metagenomics has also been used for research on epidemic of digestive infection. In an investigation of the 2011 outbreak of Shiga-toxigenic *Escherichia coli* (STEC) O104:H4, the authors tried to retrospectively identify by metagenomics the sequence of STEC in stool samples previously found positive by culture.[7] Despite a very high depth of sequencing, with about 20 millions of sequences per sample, in 13/40 cases (32.5%) the determinant gene (StxAB) was not detected.[7]

Metagenomics cannot detect some enteric pathogens. As an example, Singh et al. analysed the gut microbiota from 200 patients with enteric infections (*Campylobacter* ($n = 71$), *Shigella* ($n = 34$), *Salmonella* ($n = 66$), or STEC ($n = 28$)) firstly diagnosed by culture in comparison to 75 healthy controls. After V5-V3 16S RNA amplification, they performed pyrosequencing and demonstrated a common alteration of the gut microbiota regardless of the type of infectious agent.[8] On their OTU results, for each enteric disease, *Campylobacter* was found in only 41/71 (58,6%) patients, *Salmonella* in only 22/66 (33,3%) patients.[8] This discrepancy is linked to the depth bias, indeed, bacteria with a concentration of less than 10^6 CFU/mL are often not detected.[9]

Moreover, metagenomics analysis does not allow causative pathogen detection between many OTU. In a recent study on ventilator-associated pneumonia, authors compared metagenomic and culture for pathogen diagnosis. Without the reference method (culture), there is no possibility to know which OTU proposed by the metagenomic analysis is the causative agent.[10]

Metagenomics is insufficient to be used as a discovery tool in stools for bacteria. Only a pure culture of microorganisms enables us to understand their role with the possibility to elaborate experimental models.[4]

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Declarations

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Competing Interests

None to declare.

Ethical Approval

Not required.

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Sophie Amrane
Jean Christophe Lagier
Didier Raoult*
Aix Marseille Univ, IRD, MEPHI, IHU-Méditerranée Infection, 19-21 Boulevard Jean Moulin, 13005 Marseille, France
*Corresponding author.
E-mail address: didier.raoult@gmail.com (D. Raoult)

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"Clean care for all – it's in your hands": the May 5th, 2019 World Health Organization *SAVE LIVES: Clean Your Hands* campaign ☆

Evidence recently published in the journal confirms the importance of hand hygiene to reduce infections and cross-contamination of human pathogens in the community.[1] Quality healthcare should be available to everyone. The World Health Organization's (WHO) concept of Universal Health Coverage (UHC),[2] embodies the urgent need for access to healthcare for all people around the world. In addition to access, the concept of UHC incorporates the critical element of the necessary quality of delivered health care services. Infection prevention and control (IPC) with hand hygiene as the most effective measure, is a practical and evidence-based approach with demonstrated impact on quality of care and patient safety across all levels of the health system.

Each year, the WHO *SAVE LIVES: Clean Your Hands* campaign aims to bring people together in support of hand hygiene improvement globally on or around May 5th.[3] This year's theme for global annual hand hygiene day reflects a strong focus on providing clean care equally protecting all patients and healthcare workers from infection and antimicrobial resistance transmission, across all countries, including in low-resource settings.

WHO urges ministries of health, health facility leaders, IPC leaders, health workers, and patient advocacy groups to contribute to effective IPC action including hand hygiene as a cornerstone of quality in healthcare (Table 1). WHO invites all healthcare facilities to join the 2019 WHO Global Survey on IPC and Hand Hygiene by using two validated assessment tools; one for evaluating the core components of IPC programmes and the other for a deep dive in hand hygiene activities (https://www.who.int/infection-prevention/campaigns/ipc-global-survey-2019/en/).

On a facility level, the use of these tools gives institutions a clear understanding of the strengths and weaknesses of their IPC and hand hygiene programmes, and provides concrete actions to address existing gaps. These tools allow institutions to improve their IPC practices and policies in a concrete and measurable way, at their own speed and in their own context. The surveys are anonymous, and global results will be made available only using aggregated data. This means that facilities and ministries of health can commit fully to working on improving IPC and patient safety without fear of scrutiny or possible negative repercussions.

Globally, this survey will allow WHO to provide a situational analysis on the level of progress of current IPC and hand hygiene activities around the world and inform future efforts and resource use for IPC capacity building and improvement. Global Surveys using the Hand Hygiene Self-Assessment Framework were already conducted in 2011 and 2015,[4–6] making this year's survey even more crucial for tracking the implementation of hand hygiene and IPC on a global scale (Figure 1).

Each improvement in IPC contributes toward quality UHC. "Clean care for all – it's in your hands"!

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Alexandra Peters, Tcheun Borzykowski, Ermira Tartari
Infection Control Programme and WHO Collaborating Centre on Patient Safety, University of Geneva Hospitals and Faculty of Medicine, Geneva, Switzerland

Claire Kilpatrick, Safiah Hwai Chuen Mai, Benedetta Allegranzi
Infection Prevention and Control Global Unit, Department of Service Delivery and Safety, World Health Organization, Geneva, Switzerland

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Table 1
May 5, 2019, World Health Organization *SAVE LIVES: Clean Your Hands* campaign calls to action

Campaign participants	Call to action
Health workers	"Champion clean care – it's in your hands."
IPC* leaders	"Monitor infection prevention and control standards – take action and improve practices."
Health facility leaders	"Is your facility up to WHO infection control and hand hygiene standards? Take part in the WHO survey 2019 and take action!"
Ministries of health	"Does your country meet infection prevention and control standards? Monitor and act to achieve quality universal health coverage."
Patient advocacy groups	"Ask for clean care – it's your right."

* *IPC*, infection prevention and control

Figure 1. May 5, 2019: "Clean care for all – it's in your hands"!
The May 5, 2019, World Health Organization *SAVE LIVES: Clean Your Hands* campaign slogan and main promotional image (2019 hashtags: #HandHygiene #InfectionPrevention #HealthForAll). Campaign participants are invited to submit photos or selfies of them holding a board with the slogan and hashtags at www.CleanHandsSaveLives.org

Didier Pittet*
Infection Control Programme and WHO Collaborating Centre on Patient Safety, University of Geneva Hospitals and Faculty of Medicine, Geneva, Switzerland

*Corresponding author: Professor Didier Pittet, Infection Control Programme and WHO Collaborating Centre on Patient Safety, University of Geneva Hospitals and Faculty of Medicine, 4 Rue Gabrielle-Perret-Gentil, 1211 Geneva 14, Switzerland. Tel: +41 22 372 9828 /+41 22 372 9833 (direct).

E-mail address: Didier.pittet@hcuge.ch (D. Pittet)

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