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Letter to the Editor

In-vitro evaluation of a dual carbapenem combination against carbapenemase-producing *Acinetobacter baumannii*

Sir,

Russo and colleagues, in this Journal, recently reported a multicenter study of bloodstream infections caused by carbapenem-resistant *A. baumannii* (CRAb).[1] This underlines that the spread of CRAb is becoming a major public health concern, being listed among the top three priorities for multidrug-resistant bacteria of the World Health Organization.[2,3] It is known that almost all carbapenem-resistant *A. baumannii* produce a carbapenemase.[3] The types of carbapenemases encountered are mostly specific to the *Acinetobacter* genus.[2,3] These are mainly Ambler class D ß-lactamases possessing carbapenemase activity such as the overexpressed and naturally-occurring OXA-51 and the acquired enzymes of the OXA-23-, OXA-40- and OXA-58-types as well as the rare OXA-143.[3] The OXA-23-type enzymes are the most widespread carbapenemases in *A. baumannii*.[3] In addition, few other carbapenemases have been identified in *A. baumannii*, corresponding to the Ambler class A ß-lactamases of the KPC-type, and the Ambler class B metallo-ß-lactamases of the IMP, VIM and NDM types.[3] The CRAb clinical isolates are in their vast majority resistant to many other important antibiotics such as all ß-lactams, fluoroquinolones and aminoglycosides, making related infections challenging to treat. As opposed to carbapenem-resistant Enterobacterales, the prevalence of CRAb among *Acinetobacter* sp is quite high, ranging from 40 to 80%,worldwide.[4]

None of the novel ß-lactam/ß-lactamase inhibitor combinations currently available (ceftazidime/avibactam, ceftolozane/tazobactam, imipenem/relebactam, meropenem/ varbobactam) are efficient for treating infections due to CRAb since none of those antibiotics is active against class D carbapenemase producers. Therefore, very few therapeutic options are available currently for treating MDR *A. baumannii*,[1,5] and most of them include antibiotic combinations with polymyxins or tigecycline which efficacy remains debatable.[1] In addition, polymyxins and tigecycline molecules raise concern of poor diffusion and possible toxicity.

Early studies performed using animal models of infections or experimental treatments of humans indicate that dual-carbapenem combinations may be efficient for treating infections due to KPC and NDM producing Enterobacterales, and particularly *Klebsiella pneumoniae*.[6–10] Further in-vitro and in-vivo studies validated those findings.[6–9] The rationale of using two carbapenems (in particular ertapenem with another carbapenem) would be that ertapenem may bind to the active site of the carbapenemase with high affinity, and therefore may prevent the hydrolysis of the other carbapenem molecule. The mode of action of these dual combinations would be somehow similar to that of amoxicillin or ceftazidime combined with the ß-lactamase inhibitors clavulanic acid or avibactam, respectively.

Table 1
Evaluation of the efficacy of a dual carbapenem combination against carbapenemase-producing *Acinetobacter baumannii*.

Strain	Country of isolation	Carbapenemases	MIC (mg/L)		FIC
			IPM	MEM	
R629	France	OXA-23	64	128	0.50
R625	France	OXA-23	32	64	0.50
R628	France	OXA-23	32	32	0.75
R627	Bahrein	OXA-23	32	32	1
R622	France	OXA-23	64	64	0.75
R626	Bahrein	OXA-23	32	64	0.75
R623	Saudi Arabia	OXA-23	32	64	0.50
R624	Colombia	OXA-23	8	64	1
N715	France	OXA-23	16	16	0.75
N688	Switzerland	OXA-23	32	32	1
N690	Switzerland	OXA-23	64	64	0.50
R703	Brazil	OXA-40	64	64	0.60
R704	France	OXA-40	64	64	0.60
R824	Turkey	OXA-58	32	64	0.50
R825	Romania	OXA-58	32	8	0.75
N7	Switzerland	OXA-72	64	128	0.75
N420	Switzerland	NDM-1	8	8	0.60
N15	Switzerland	NDM-1	32	32	0.75
R32	France	NDM-1	32	32	0.60
R34	France	NDM-1	16	64	0.50
N655	Switzerland	NDM-1	64	64	1
N739	Switzerland	NDM-5	128	64	0.60
R71	France	IMP-4	16	32	0.75

IPM: Imipenem; MEM: Meropenem.

To the best of our knowledge, dual carbapenem combinations have never been tested against carbapenemase-producing *A. baumannii*. Therefore, our goal was to evaluate the in-vitro activity of a dual carbapenem combination, i.e. imipenem and meropenem, against *A. baumannii* isolates producing different types of carbapenemases. Imipenem and meropenem were retained since those carbapenems are widely used for treating infections caused by *A. baumannii*. Ertapenem was not tested since *A. baumannii* is naturally resistant to ertapenem, likely related to its weak penetration through the outer membrane.

A collection of twenty three non-clonally related *A. baumannii* isolates recovered from clinical samples from Barhein, Brazil, Colombia, France, Saudi Arabia, Switzerland, and Turkey was tested. All the isolates were non susceptible to imipenem and meropenem (Table 1). Following the latest EUCAST breakpoints (http://www.eucast.org/), isolates with MIC values of imipenem and meropenem $\leq$ 2 mg/L are categorized as susceptible, whereas those with MICs of imipenem >4 mg/L or and MIC of

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meropenem >8 mg/L are considered resistant. The nature of the carbapenemase produced by each isolate was characterized at the molecular level. Our collection included eleven OXA-23, two OXA-40, two OXA-58, one OXA 72, six NDM and one IMP producers that mirrors the variety of acquired carbapenemases identified in *A. baumannii* and their distribution worldwide (Table 1). Fractional inhibitory concentration (FICs) index were calculated according to the following formula, ΣFIC= FIC of imipenem + FIC of meropenem where FIC of imipenem or meropenem = MIC of imipenem or meropenem in combination, divided by the MIC of https://www.ncbi.nlm.nih.gov/pubmed/?term=NDM+egypt imipenem or meropenem alone. Interpretation of the results was based on the following; FIC values of ≤ 0.5 indicate synergy, FIC values of >0.5 to 4 indicate no interaction, and FIC values >4 indicate antagonism.[11]

Six out of the twenty three isolates tested showed synergy activity of imipenem and meropenem (ca. 25%) whereas the combination of imipenem and meropenem were indifferent for the other tested carbapenemase-producing isolates. Among the six isolates for which a synergy was identified, different types of carbapenemases were produced. No antagonism between imipenem and meropenem was identified for any of the isolates.

The rate of in-vitro synergy observed between imipenem and meropenem for those CRAb corresponds to that identified for carbapenemase-producing *K. pneumoniae*.[11] In addition, we identified here a synergy for a single *A. baumannii* isolate producing NDM-1 whereas that synergy as determined by the results of th FIC values was not identified for any of the NDM-1-producing *K. pneumoniae* tested previously.[11]

Our study suggests that dual carbapenem combination may be effective, to some extent, against some carbapenemase-producing *A. baumannii*. This dual combination may therefore work in some cases to inhibit not only the activity of carbapenemases of class A (KPC) and class B (NDM) as known in Enterobacteriales. but also of those peculiar class D carbapenemases specific to *A. baumannii* (OXA-23, OXA-40 and OXA-58). Due to the dearth of novel antibiotics for treating infections due to MDR *A. baumannii,* this dual carbpenem combination may offer an alternative since all MDR *A. baumannii* produce a carbapenemase. By targeting the inactivation of the carbapenemase, we are targeting actually the MDR pattern of those strains.

Further investigations using animal models of infection and clinical trials are required to further establish this treatment as an alternative against MDR *A. baumannii*.

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Declaration of Competing Interest

None to declare.

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Clinical and epidemiological characteristics of Coxsackievirus A6- and Enterovirus 71-associated clinical stage 2 and 3 severe hand, foot, and mouth disease in Guangxi, Southern China, 2017

Dear Editor,

We have recently reported in this journal that six amino acids of VP1 switch along with pandemic of CV-A6-associated HFMD in Guangxi, southern China, 2010–2017.[1] HFMD, a common childhood infectious disease, has become a global public health issue,

especially in the Asia-Pacific region. The symptoms of HFMD are generally mild, which include fever, blisters on the hands and feet, and ulcers inside or around the mouth. However, some infectors or developed serious neurological or systemic complications, even fatal. In order to improve early recognition of severe cases, which is utmost important in diagnosis and treatment of patients with HFMD, a new Chinese guidelines for the diagnosis and treatment of HFMD (2018 edition) was issued in 2018.[2] Timely and accurate recognition of stages 2 and 3 of HFMD is critical for the diagnosis and treatment of severe cases.

Besides EV-A71 and Coxsackievirus A16 (CV-A16), CV-A6 has been a major, even dominant pathogen for HFMD in China mainland since 2013. Moreover, the percent of severe cases caused by CV-A6 has gradually increased in recent years.[3] However, the detail for the association between clinical features and pathogens, especially for severe HFMD cases, are still need to be explored.

The Guangxi autonomous region in southern China has been one of the top three provinces most affected by HFMD in China since 2008.[4] In Guangxi, severe HFMD cases reported in 2017 had highly increased compared with those in 2015 or 2016.[1] However, the clinical, epidemiological, and pathogenic characteristics of these severe HFMD cases are still unknown. This study focused on characterizing major clinical aspects and the pathogen spectrum of severe HFMD cases in Wuzhou city, with the proportion of severe cases reported accounting for as high as 70.20% of total severe cases in Guangxi, 2017.

HFMD case-reporting criteria was based on the national guidelines for the control and prevention of HFMD (issued by the Ministry of Health in China (2009)). The diagnosis criteria for HFMD and laboratory test procedures was followed the HFMD Clinical Diagnosis and Treatment Guidelines (2018 edition). There are five HFMD clinical stagings, including stage 1 (Eruption), stage 2 (Nervous system involvement), stage 3 (Early cardiopulmonary failure), stage 4 (Cardiopulmonary failure), and stage 5 (Recover). Patient of clinical stage 1 belongs mild case and stage 2–4 are severe cases. Clinical specimens (stool, rectal swabs or throat swabs) were collected and then Enterovirus were detected. All analyses were performed using R (R Foundation for Statistical Computing, Vienna, Austria; https://www.r-project.org/). The p values from Fisher's exact test were adjusted for multiple testing with BH method. In the 2 by 2 cases, greater and less alternative hypothesises were tested and adjusted respectively, and the smaller q values for each comparison was employed.

The Guangxi autonomous region is located in southern China and Wuzhou is a city adjacent to Guangdong province (Fig. S1A,B). There were a larger amount of reported cases as well as severe cases in 2017 compared with 216 in wuzhou city (Fig. S1C,D). A total of 23,075 clinical cases, with 2615 severe cases and 1 fatal case, were reported in Wuzhou city from January to December 2017. Non-EV-A71 and CV-A16 enterovirus become the dominated pathogens in 2013, 2015, and 2017 (Fig. S1E).

From January to December 2017, 2350 severe HFMD cases, with 2194 clinical stage 2 and 156 clinical stage 3 cases in wuzhou city were enrolled in this study. The major clinical features of these severe cases were included respiratory tract infection and neurological symptoms. Poor mental condition, rash on hand, foot, and mouth, and fever were the top three most frequent symptoms for both stage 2 and 3 cases (Fig. 1A). Comparing with stage 2 cases, nine symptoms occurred more frequently in stage 3 cases, such as rash on hand, foot, and mouth, fever, startle, rash on buttock, arms or legs, trunk, heart rates >120, vomiting, limb tremors, twitching, and ecphysesis (all $q < 0.05$). Meanwhile, poor mental condition and dysphoria were happened less frequently in stage 3 cases than stage 2 cases ($q < 0.05$).

There was a network representing co-occurrence of clinical features in the patients with HFMD clinical stage 2 or 3. The red lines indicated significantly positive correlations while the green lines represented significantly negative correlations ($q < 0.05$) (Fig. 1B). For stage 2 cases, poor mental condition and rash or rash on hand, foot, and mouth were the most common paired signs (93.97%, and 93.51%, respectively), followed by dysphoria and poor mental condition or fever (82.29%, and 73.06%, respectively). The frequencies for paired signs of startle and rash or rash on hand, foot, and mouth were 60.96% and 60.92%, respectively. In contrast, for stage 3 cases, fever and startle were the most common paired signs (75.66%), followed by heart rates >120 and startle or rash on buttock, arms or legs, trunk (47.69%, and 37.86%, respectively).

The stage 2 cases occurred all the year round with two peaks during April to June and August to October (Fig. 1C). While the stage 3 cases occurred during March to September. The average age and the median age of onset were 22-month and 18-month old for stage 2 cases, 21-month and 16.5-month old for stage 3 cases, respectively. Age distribution showed that children under 5 years old accounted for more than 90% of stage 2 or 3 cases (Fig. 1D). We found 13 to 24-month-old represented the highest incidence in both stage 2 and 3 cases, and then, 7 to 12-month-old represented the second highest incidence of these severe cases. There was no significantly difference on the age distribution between stage 2 and 3 cases ($p = 0.2004$).

RNA extraction and detection of pathogens (pan-EV, EV-A71, CV-A16, C-A6 and CV-A10) detection was performed as previously described.[4] Sample with positive for pan-EV but negative for EV-A71, CV-A16, C-A6 and CV-A10 was considered to be other EV positive. A total of 1704 HFMD cases were positive for enteroviruses, including 635 cases (37.26%) positive for CV-A6, 621 (36.44%) positive for EV-A71, 236 (13.84%) positive for CV-A10, 4 (0.23%) positive for CV-A16, and 141 (8.27%) for other EV. For clinical stage 2 cases, the dominated pathogen was CV-A6 ($n = 298$, 45.71%), followed by EV-A71 ($n = 176$, 26.99%) (Fig. 2A). However, the predominated pathogen became EV-A71 ($n = 17$, 45.95%), and the second causative agent was other EV ($n = 9$, 24.32%) in clinical stage 3 cases (Fig. 2B). The percents of CV-A10 positive cases were similar both in stage 2 and 3 cases (16.10% and 16.22%), while the proportion of EV-A71, CV-A6 and other EV were variant between stage 2 and 3 cases.

In our study, EV-A71 was prevalent at June to September, with a peak at July for stage 2 cases and two peaks at July and September, respectively, for stage 3 cases. In contrast, CV-A6 occurred during July to November, with a shark peak at October for stage 2 cases, and with a small peak at August for stage 3 cases (Fig. 2C-D). Meanwhile, CV-A10 and other EV mainly presented at July to October. The age distribution among different pathogens were similar ($p = 0.092$), that was 13 to 24-month-old represented the highest incidence and followed by 7 to 12-month-old (Fig. 2E).

We wonder whether different pathogens contribute to different symptoms, so we analyze the symptom among four key pathogens. For clinical stage 2 cases, compared to CV-A6 positive cases, EV-A71 and CV-A10 positive cases had higher incidence of startle (71.75%, 65.71%, and 40.94%, respectively, $q = 0.000$), heart rates >120 (24.86%, 27.62%, and 15.44%, respectively, $q = 0.0142$) (Fig. 2F). While CV-A6 positive cases had higher frequency of convulsions (8.05%, 0.56%, and 2.86%, respectively, $q = 0.0048$) than EV-A71 and CV-A10. For clinical stage 3 cases, there was no significant difference between different pathogens.

Then the clinical symptom preference of pathogens were explored (Fig. 2G). For clinical stage 2 cases, CV-A6 tend to cause more coughing and convulsions, and less symptoms of rash on hand, foot, or mouth, startle, and heart rates >120. In contrast, EV-A71 and CV-A10 tend to contribute startle. In addition, EV-A71 tend to cause less symptoms of coughing and rash on buttock, arms or legs, trunk.

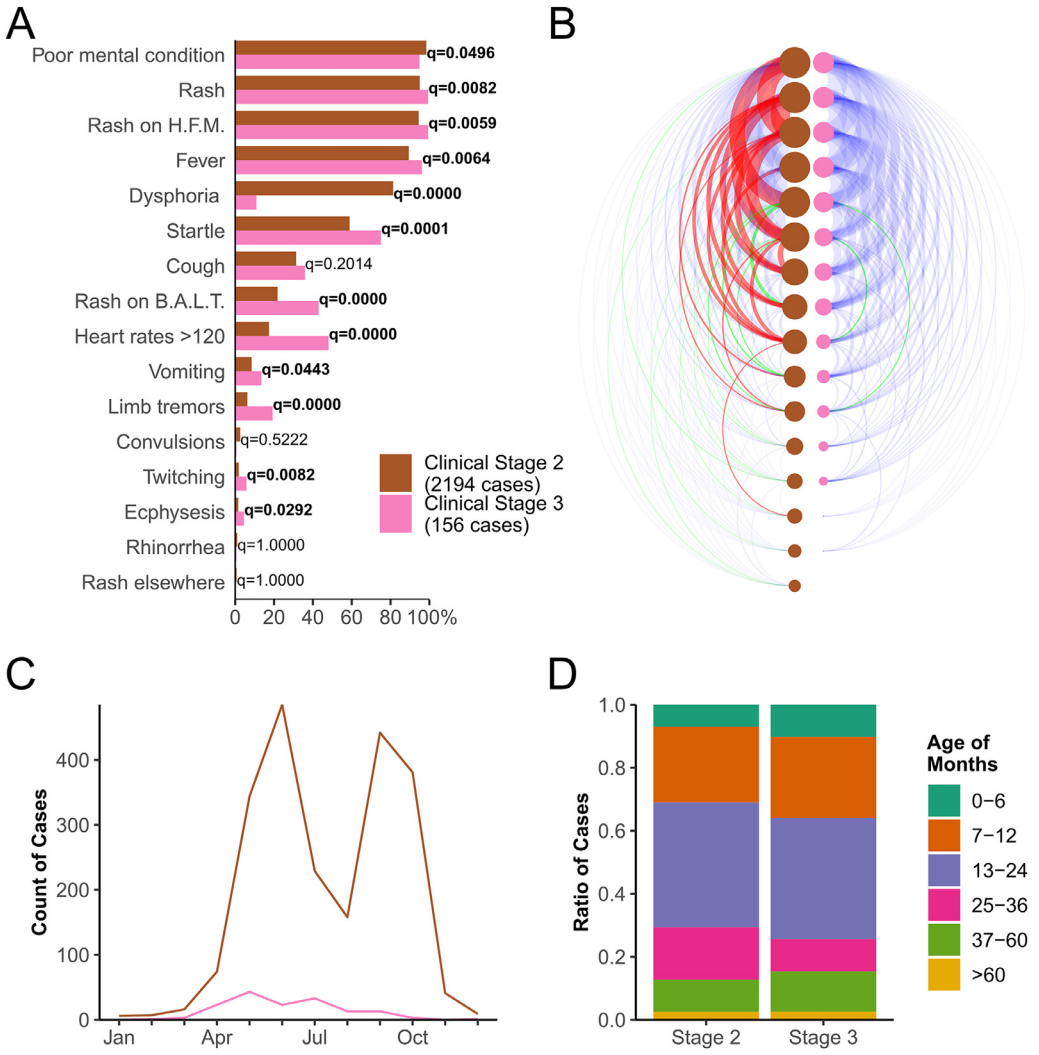

Fig. 1. The arc diagram of symptoms and signs of patients with stages 2 and 3 severe HFMD (A-B). The red lines indicated significantly positive correlations while the green lines represented significantly negative correlations ($q < 0.05$). Monthly distribution and age distribution of stages 2 and 3 cases (C-D).

There are five clinical stages according to the Chinese newest diagnosis and treatment criterion (2018 edition), with stage 1 considered to be mild cases and stage 2–4 to be severe cases. Subdividing the stages of severe cases are distinctly helpful for diagnosing and preventing from developing critical illness and even death. Among 9 symptoms happened more frequently in stage 3 than stage 2, rash on buttock, arms or legs, trunk, heart rates >120, limb tremors, twitching, and ecphysesis are at least two fold higher happened in stage 3 cases, which may facilitate clinical diagnosis.

In our study, the proportion of fever, cough, vomiting and dysphoria in stage 2 and 3 severe cases are much higher than those for mild cases.[5] Relatively mild neurological symptoms, as well as hardly any respiratory and circulatory symptoms of severe cases are observed in clinical stage 2 and 3 cases. There are more neurological, respiratory and circulatory symptoms occurred in severe cases in previous study [6] than our study, in which more stage 3 and 4 cases were enrolled, than our study.

Our findings are consistent with previous results. One of similar results is the incidence of rash on hand or foot among CV-A6-associated cases was significantly lower than EV-A71-associated cases. The second similar results is the incidence of twitching, which is higher in CV-A6-associated cases than EV-A71-associated cases.[6] There is also no significant difference in the incidence of vomiting and cough in our study and previous results.

For stage 2 cases, beside rash, the incidences of startle and heart rates >120 are also lower in CV-A6-associated cases than EV-A71-associated cases or CV-A10-associated cases. In contrast, the incidence of convulsions and limb tremors are higher in CV-A6-associated cases than EV-A71-associated cases. Thus, the differences in symptoms preference may exist in different pathogens and it is still needed further exploration.

There are significant difference for mild cases in age distribution between EV-A71 and CV-A6,[7] but no difference for severe cases in our study and previous finding.[8] The monthly distribution of pathogens are total different. EV-A71 are mainly occurred in summer (April to July) most CV-A6 circulated at autumn (August to November).[1,6,8]

Recently, CV-A6 and CV-A10 have become major pathogens not only for mild cases,[1,9] but also for severe cases.[3] Overall, EV-A71 and CV-A6 became the dominant pathogens for clinical stage 2 and 3 HFMD cases, with mild neurological symptoms, in Guangxi, southern China, 2017. This study provides further insights into the relationship between clinical features and pathogens of severe HFMD, and it will be helpful for HFMD diagnosis and pathogenic surveillance.

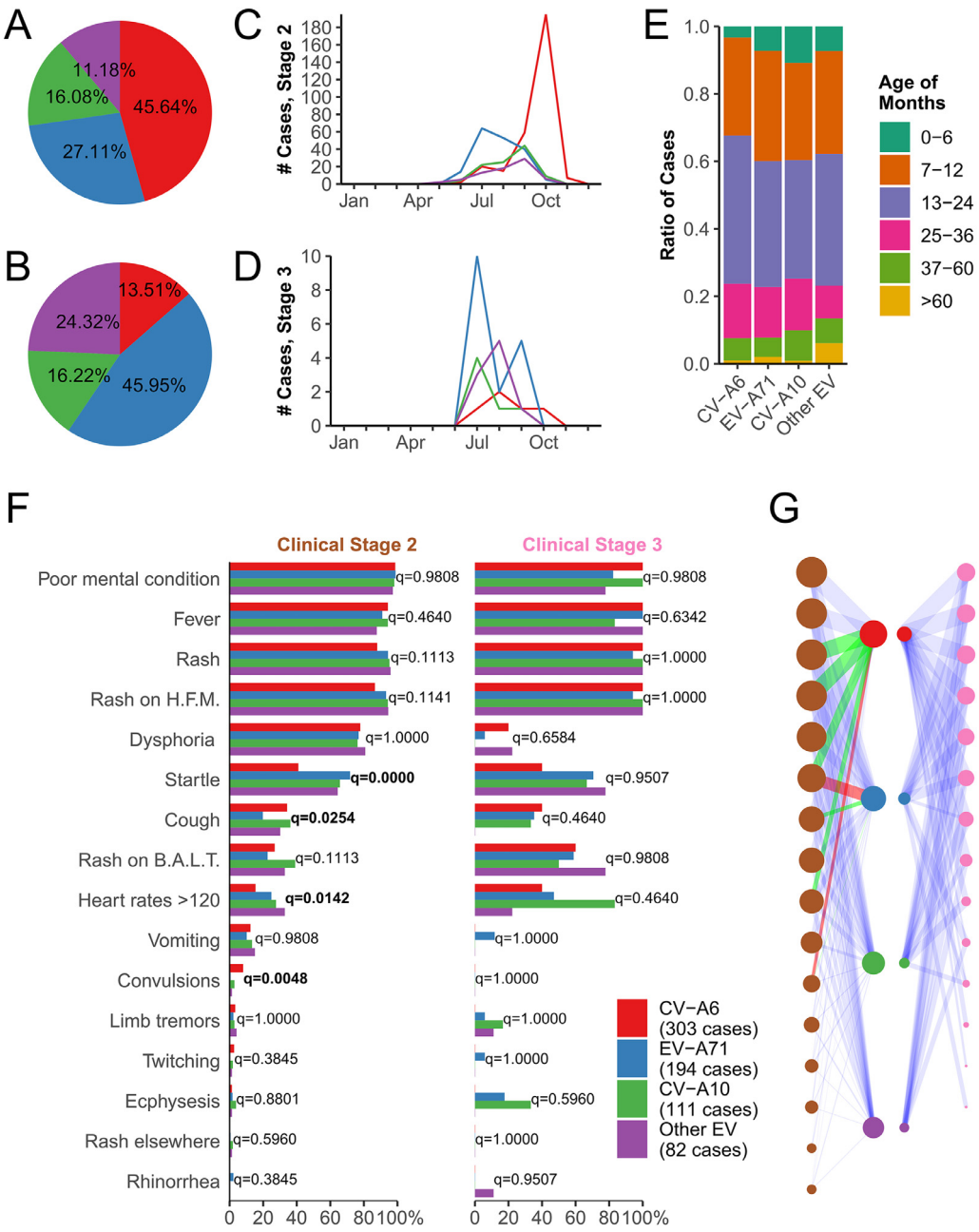

Fig. 2. The EV genotypes of stages 2 and 3 cases in wuzhou, 2017 (A-B). Monthly distribution (C-D) and age distribution of different pathogens (E) for stages 2 and 3 cases. The clinical features of stages 2 and 3 cases for different pathogens (F) and the potential symptoms preference of different pathogens (G). The red lines indicated significantly positive correlations while the green lines represented significantly negative correlations ($q < 0.05$).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2019.09.021.

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Carbapenem/β-lactamase inhibitor combination in complicated urinary tract infection

Dear Editor,

We read with great interest two articles[1,2] reported the treatment of urinary tract infection caused by multi-drug resistant organism (MDRO). First one showed inappropriate empirical antibiotics and KPC carbapenemase-producing *Klebsiella pneumonia* could be associated with increased risk of treatment failure in UTI due to *K. pneumoniae*.[1] Second one demonstrated the high efficacy of colistin for the treatment of complicated urinary tract infection (cUTI) caused by extremely drug resistant *Pseudomonas aeruginosa*, however, the risk of nephrotoxicity remained a serious concern.[2] Actually, therapeutic option in the treatment of cUTI caused by MDRO is limited,[3–5] and it is an urgent issue to develop an novel agent which is effective and safe in this difficult clinical condition. Recently, the development of novel carbapenem/β-lactamase inhibitor combination - imipenem-relebactam and meropenem-vaborbactam may be one of resolution. In 2019, three phase 3 randomized studies -TANGO I, TANOG II and RESTORE-IMI 1 investigating the clinical efficacy and safety of these novel carbapenem/β-lactamase inhibitor combinations was published.[6–8] Therefore, we extracted the findings of patients with cUTI/acute pyelonephritis (APN) from these three studies and conducted an integrated analysis to evaluate the usefulness of -imipenem-relebactam and meropenem-vaborbactam.

TANGO I compared the clinical efficacy and tolerability of meropenem-vaborbactam or piperacillin-tazobactam in the treatment of adult patients with cUTI/APN.[6] TANGO II compared the effect and safety of meropenem-vaborbactam and best-available therapy (mono- or combination therapy with polymyxins, carbapenems, aminoglycosides, tigecycline; or ceftazidime-avibactam alone) in the treatment of adult patients with confirmed/suspect carbapenem-resistant Enterobacteriaceae (CRE) infections.[8] RESTORE-IMI 1 compared the usefulness of imipenem-relebactam and colistin plus imipenem in patients with imipenem-nonsusceptible bacterial infection.[7]

In this study, we used microbiologic modified intent-to-treat (mMITT) population included all cUTI/APN patients in the modified intent-to-treat (MITT) population had a baseline qualifying bacterial pathogen to assess the overall response. In addition, we used the MITT population comprised all patients with all type of infections who received 1 or more doses of study drug to evaluate the risk of adverse events.

First, there was no significant difference in terms of overall response at test of cure (TOC) between carbapenem/β-lactamase inhibitor combination and comparator (risk ratio [RR], 0.99, 95% CI, 0.82–1.20) in the pooled analysis of three trials.[6–8] Second, only two studies[6,8] reported the overall response at end of treatment (EOT) and the pooled analysis showed that carbapenem/β-lactamase inhibitor combination was better than comparator (RR, 1.05, 95% CI, 1.01–1.10). Third, only TANGO I and II trials[6,8] reported the clinical response rate at EOT and TOC, and the pooled analysis showed that carbapenem/β-lactamase inhibitor was associated with similar clinical response rate than and comparator at EOT (RR, 1.05, 95% CI, 1.01–1.09) and TOC (RR, 1.05, 95% CI, 0.98–1.13). Forth, microbiological eradication rate was reported in TANGO I[6] and RESTORE-IM 1,[7] and carbapenem/β-lactamase inhibitor was noninferior to comparator in the terms of microbiological eradication rate at EOT (RR, 1.06, 95% CI, 1.02–1.11) and TOC (RR, 0.98, 95% CI, 0.70–1.38). Finally, the safety analysis of three trials[6–8] showed that carbapenem/β-lactamase inhibitor combination had a similar risk of i) treatment emergent adverse events (TEAEs) (RR, 0.96; 95% CI, 0.81–1.14), ii) any serious adverse events (RR, 0.68; 95% CI, 0.33–1.43), iii) discontinuation of study drug due to TEAE (RR, 0.51; 95% CI, 0.23–1.13), iv) drug-related TEAE (RR, 0.76; 95% CI, 0.42–1.40), v) death (RR, 0.72, 95% CI, 0.35–1.50) when compared with the control group. In contrast, carbapenem/β-lactamase inhibitor combination was associated with lower risk of and treatment emergent nephrotoxicity (RR, 0.25; 95% CI, 0.08–0.78) than comparator.

In conclusion, carbapenem/β-lactamase inhibitor combination exhibits comparable efficacy with comparator and is tolerable in cUTI/APN, so it can be recommended as one potential therapeutic option in this critical condition.

Declaration of Competing Interest

None.

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Listeria monocytogenes in *human milk* in *Mali:* A *potential health emergency*

In this Journal, Scobie and colleagues recently emphasized the continuing high mortality associated with listeriosis.[1] Most cases occurred in people over 60 years of age (74%) with only a few cases in children (1%). However, this pattern is expected to be different in least developed countries, such as Mali. While using microbial culturomics and 16S amplicon sequencing approaches to decipher the human milk microbiota in Mali and France, we serendipitously isolated *Listeria monocytogenes* from healthy Malian mothers living in an area endemic for severe acute malnutrition.

Perinatal listeriosis represented one fifth of all cases (21%) and has been associated with amnionitis, leading to respiratory symptoms, meningitis and death among newborns.[2] While placental infection has been well documented, maternal milk has been neglected as a potential source of *L. monocytogenes* infection and prevalence in human milk has not been investigated.[1–3] Although one transmission from mother's milk to her baby with neonatal infection has been reported,[4] it is not known to be part of the human milk microbiota repertoire.[3]

Over the past 10 years, we have developed a polyphasic approach to the study of the human microbiota by combining culturomics and 16S amplicon sequencing,[5] two approaches that exclude *a priori* hypotheses and identify unexpected outbreaks. To explore the milk microbiota diversity across geography, ten healthy lactating mothers were recruited in Mali (at Kalabancoro Vaccination and Healthy Children Surveillance Unit, Kalabancoro, a suburb of Bamako), which is a known area with high prevalence of children suffering from severe acute malnutrition) and 144 in Marseille (neonatology unit of the Hôpital de la Conception and Hôpital Nord). Milk samples were collected in sterile tubes by manual pressing after skin cleaning with sterile water and antiseptic agent. Written consents have been obtained and the study was approved by the local ethics committee in France and in Mali. In the present study, two conditions were used in relation to the small volume, including incubation in blood culture bottle with rumen and sheep blood, in aerobic and anaerobic atmosphere at 37 °C.[5] All phenotypic analyses of colonies isolated in this study were performed by matrix assisted laser desorption ionization–time of flight (MALDI-TOF). All samples were also analyzed by v3v4 16S amplicon sequencing as previously reported.[6] The specific *L. monocytogenes* PCR targeting the *hly* gene was directly performed on specimen as previously described.[7] In parallel, specimens were enriched in Fraser broth and incubated during 24 h at 37 °C and 48 h at 4 °C. Broths were then subcultured on Columbia agar and PALCALM medium at 37 °C. Specimens were also directly inoculated onto solid PALCAM medium (at 37 °C and 4 °C). Species identification was confirmed by genome sequencing and analysis of the 16S and rpoB gene, digital DNA-DNA hybridization, and average nucleotide identity as previously described.[8] The assembly of the strains was performed with Spades and annotated with Prokka. Average nucleotide identity was calculated using orthoANI (http://www.ezbiocloud.net/sw/oat). The pangenome of the 8 strains isolated in this study and the representative genome of L. *monocytogenes* (National Center for Biotechnological Information) was performed according to the presence/absence of genes using Roary.

L. monocytogenes was detected in all the 10 included mothers in Mali but in none of the 144 French mothers. Using non-specific methods, 9/10 were positive by culturomics and 6/10 by v3v4 16S amplicon sequencing. We confirmed the presence of viable *L. monocytogenes* in milk samples from Mali using specific culture and PCR. 4/10 samples were positive using Fraser broth medium subsequently seeded on PALCAM medium, 4/10 with direct inoculation on PALCALM medium, and 9/10 by quantitative PCR. Two strains isolated by culturomics and six strains isolated by specific culture were available for genomic analysis, and the digital DNA-DNA hybridization (dDDH) showed that the closest species was *L. monocytogenes* with an 87.3% dDDH value. Average nucleotide identity (OrthoANI values) between our strains ranged from 99.9 to 100%, and 98.5–98.6% with *L. monocytogenes* (Fig. 1a and b). OrthoANI values were much lower for other *Listeria* species (<90%, Fig. 1a). Therefore, the genomic analysis demonstrated that the 8 strains recovered in this study were closely related and corresponded to the *L. monocytogenes* species. The pangenome

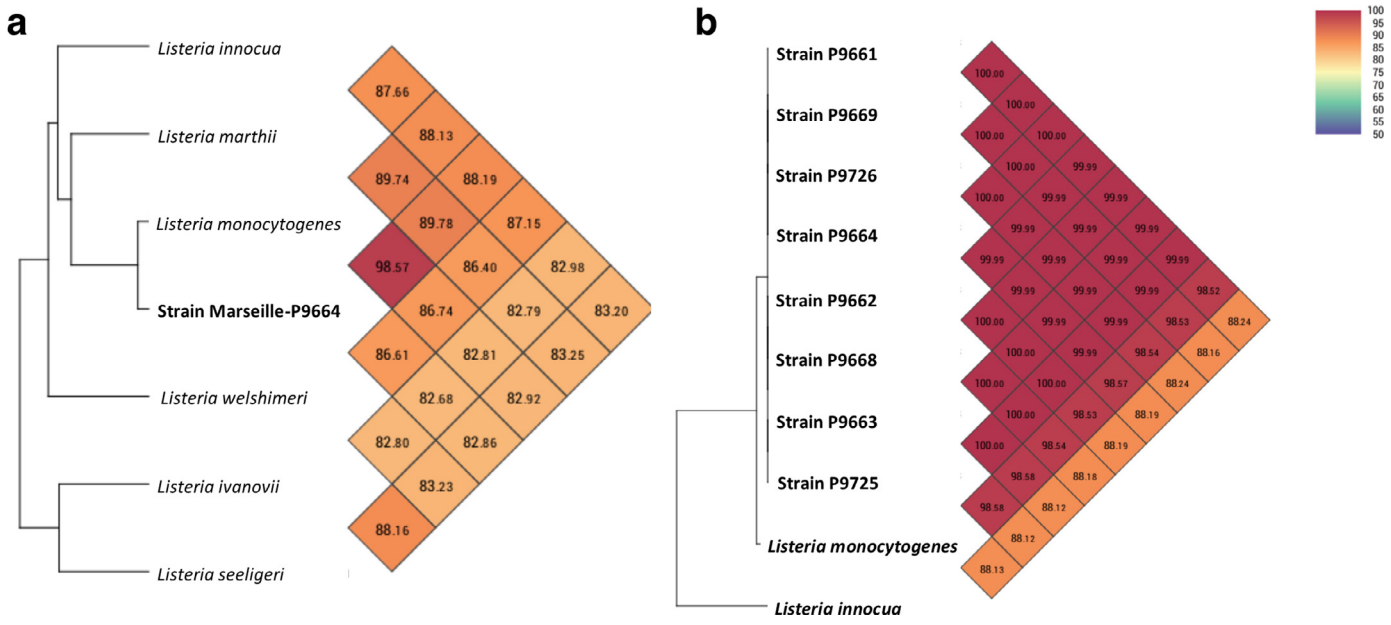

Fig. 1. Genomic analyses of *L. monocytogenes* isolated from human milk in Mali. Genomic analysis confirmed that all 8 strains were closely related and corresponded to *L. monocytogenes* (average nucleotide identity of orthologous fragment pairs calculated with OrthoANI).

analysis including our 8 strains and the type strain confirmed that the type strain formed an outgroup. We therefore investigated if all the strains isolated here correspond to a clonal dissemination. All but two strains (P9669 and P9726) harbored a plasmid (59 genes, 62,000 nucleotides), and the results of the accessory genome analysis were in support of polyclonality.

These results suggest that *L. monocytogenes* could be part of the human milk microbiota, notably in least developed countries endemic for severe acute malnutrition. This was confirmed by the culture of several strains, specific culture, specific PCR, genome sequencing and most recent taxonomic methods. Microbial culturomics[5] was critical in this discovery because it was the most sensitive approach (9/10 – equal to quantitative PCR and better than v3v4 16S amplicon sequencing) and has the unbeatable advantage to obtain strains that can be stored, sequenced and fully characterized. In a recent comprehensive literature analysis, we found only one case reporting *L. monocytogenes* in human milk associated with neonatal infection.[3,4] Transmission of *L. monocytogenes* in mammal milk is well known but has not been investigated in humans. The prevalence of *L. monocytogenes* is probably neglected in least developed countries endemic for chronic diarrhea and malnutrition because *L. monocytogenes* is not systematically sought in this context. Since severe acute malnutrition and chronic diarrhea are intimately intricated,[9] it is possible that digestive contamination through breastfeeding in newborns and infants can contribute to neonatal listeriosis, chronic diarrhea and malnutrition in children. Confirmation of the transmission from mother to child through human milk represents a unique and unexpected opportunity to understand and manage a possible hyperendemicity of *L. monocytogenes* in the least developed countries, thus contributing to their development.

Declaration of Competing Interest

The authors declare no competing interests.

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Ethical consideration

Written consent was obtained from each mother before sampling in accordance with the Helsinki declaration and CIOMS 2016. The study and consent procedure were approved by the ethics committee of IFR 48 under consent number 2016–004 and by FMPOS Institutional Ethics Committee (Mali, CE-FMPOS) under number 2014/46/CE/FMPOS as of May 22, 2014 (available on request). The material transfer agreement is available upon request. The samples were transferred from Mali to France in accordance with the Nagoya protocol.

Data deposition

The 8 strains were deposited in our culture collection (Collection de Souches de l’Unité des ricketssies) under numbers P9661, P9662, P9663, P9664, P9668, P9669, P9725, and P9726. The 8 corresponding genomes were deposited under the Bioproject PRJEB32287.

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Cystic and alveolar echinococcosis are two completely different diseases caused by two different species of *Echinococcus* parasites. comment ON: Disseminated cystic echinococcosis of Ferdinando II de' Medici, Grand Duke of Tuscany (1610–1670) by Gaeta R, Giuffra V. J infect. 2019 Sep 4

Sir,

We read with attention the Letter to the Editor published by Raffaele Gaeta and Valentina Giuffra accepted for publication in the September 2019 issue of Journal of Infection.[1] We were very surprised that the authors stated: "The picture described by the doctors is a typical case of disseminated cystic echinococcosis, a parasitic disease caused by tapeworms *Echinococcus multilocularis*".

Cystic echinococcosis (CE) and alveolar echinococcosis (AE) are two completely different diseases, caused by two different species of parasites of the genus *Echinococcus* (*E. granulosus* for CE and *E. multilocularis* for AE), with different life cycles and different geographical distribution (although overlapping in some areas, especially China).[2]

Cystic echinococcosis (CE) is an infection caused by the cystic larval stage of the parasite *Echinococcus granulosus sensu lato (s.l.)*.[3] *E. granulosus s.l.* is naturally transmitted between canids, especially the domestic dog, and livestock, especially sheep but also goats, pigs, cattle, and camelids. *E. granulosus s.l.* is distributed worldwide where livestock raising is practiced, including the Mediterranean and Italy.[2] The cysts of *E. granulosus s.l.* in humans may develop in any organ, primarily affecting the liver and lungs; the majority of CE cases are asymptomatic or pauci-symptomatic and the disease has mostly a chronic, often rather benign development. Symptoms and complications may derive from compression on neighbouring structures, superinfection, and rupture, and disseminated cystic echinococcosis is well described.[4,5] Cysts of *E. granulosus s.l.* develop as well-defined liquid-containing cysts, of various size, growing concentrically.[2]

E. multilocularis, on the contrary, is the causative agent of alveolar echinococcosis (AE).[6] *E. multilocularis* is endemic only in the northern hemisphere and its life cycle is mainly sylvatic, developing mainly between foxes and other wild canids and small rodents.[2] In Italy, foxes infected with *E. multilocularis* have been reported only in Trentino Alto-Adige, representing the southern edge of the parasite distrbution in Europe, probably because of the distribution of competent alpine rodent intermediate hosts.[7]

The morphology of *E. multilocularis* lesions and AE disease behaviour are completely different from *E. granulosus s.l.* cysts and CE. AE is a devastating disease primarily affecting the liver, with malignant tumour-like development and fatal outcome if not treated.[4,5] The metacestode of *E. multilocularis* has a multicystic appearance, forming infiltrative lesions with necrotic areas.[6]

The statement "disseminated cystic echinococcosis […] caused by tapeworms Echinococcus multilocularis" is evidently a contradiction in terms. The description of the lesions reported by the authors from the necropsy documents of Ferdinando II de' Medici, their distribution, as well as the geographical area (Tuscany) where Ferdinando II was born and lived, are consistent with CE possibly being the described disease, which is caused by *E. granulosus s.l.*

All efforts should be made by the scientific and medical community to overcome the long lasting problem of discussing CE and AE together, evidently fostering confusion and imprecision, and of heterogeneous and ambiguous reporting of these two entities in official reports.[8,9]

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Metagenomic data screening reveals the distribution of mobilized resistance genes *tet*(X), *mcr* and carbapenemase in animals and humans

Dear Editor,

In March 2019, The Journal of Infection published our finding[1] that live poultry markets (LPMs) is a huge antibiotic resistance gene (ARG) reservoir, including the plasmid-mediated colistin resistance gene *mcr-1*, which have been in the human gut for a long time.[2] Recently, a novel mobilized *mcr-9* in a multidrug-resistant (MDR) *Salmonella enterica* Serotype Typhimurium isolate was reported.[3] The Gram-negative bacteria co-producing *mcr* genes and carbapenemases limits our choice for treating MDR infections, however, tigecycline has been considered as the last-resort antibiotic. Although tigecycline has never been used in animal husbandry in China, two unique mobile tigecycline resistance genes, *tet*(X3) and *tet*(X4), were firstly discovered in bacteria isolated from pigs.[4] Thus, we downloaded metagenome datasets[1,5–10] available and did a retrospective study to reveal the distribution of the newly discovered *tet*(X), *mcr* and carbapenemases (Table S1) in human and animal gut microbiomes.

We were surprised to find that a gene with 100% nucleotide identity with the *mcr-9* gene. It is noteworthy that *mcr-9* was the only one identified in human gut samples (1.4%, 28/2019). Importantly, *mcr-9* was present in one American ($n = 139$), nine Chinese ($n = 368$), and twelve European ($n = 760$) gut microbiomes (Table 1 and Fig. 1A). Moreover, *mcr-9* was identified in two Indians ($n = 110$), two adults from the UK and two Swedish infants ($n = 98$) (only four months, Fig. 1A and Table 1). Interestingly, the *mcr-9* gene was not detected in the two Swedish children mother. As some of these *mcr-9*-harboring gut samples were collected before 2011, it means that the worrisome spread of *mcr-9* might have been in the human gut for a long time and the dissemination of these genes has long been undiscovered. Although *mcr-9* is a mobilized resistance gene, it can be detected in both plasmids and chromosome genomes (identity=100%) by searching *mcr-9* in 14,764 complete bacteria genomes (accessed 26 July 2019). Among these samples, four human samples carried both the *mcr-1* and *mcr-9* genes. The *mcr-1* gene was also identified in 27 human gut samples collected from China, 71 chicken gut samples collected from poultry farms in China and 71 poultry faecal samples collected from China LPMs[7] (Fig. 1B and Table 1), including chicken ($n = 56$), duck ($n = 12$), geese ($n = 3$). Our results illustrated that the number of *mcr-1*-carrying samples was more in animals (15.6%, 142/912) than in humans (1.3%, 27/2019). Moreover, the *mcr-3* gene was detected in four poultry faecal samples from China LPMs. Raw reads mapping identified *mcr-4* and *mcr-5* gene fragments in one and two poultry gut samples, respectively.[1] The plasmid-mediated colistin resistance has already spread in the gut microbiomes of both human and animal worldwide,[1,2] as occurred with NDM-1 (New Delhi metallo-beta lactamase-1) nine years ago. Thus, it would be interesting to see where *mcr* genes were from. We subsequently assessed the prevalence of NDM in both human and animal gut microbiomes. Interestingly, NDM-5 was identified in one Indian ($n = 110$) and 40 chicken samples ($n = 495$) from China. NDM-1 gene fragments were detected in poultry feces from LPMs in China by raw reads mapping.[1]

We also found that tigecycline resistance gene *tet*(X3) was present in 33 poultry gut microbiomes (identity=100%) of 130 poultry samples from China LPMs, including 22 chickens, eight ducks, two geese, and one pigeon (Table 1). These *tet*(X3)-carrying samples were found in twelve provinces in China (Fig. 1B and Table S2): Anhui ($n = 1$), Fujian ($n = 5$), Henan ($n = 1$), Hunan ($n = 1$), Jilin ($n = 1$), Jiangsu ($n = 5$), Jiangxi ($n = 3$), Inner Mongolia ($n = 1$), Qinghai ($n = 3$), Shandong ($n = 3$), Yunnan ($n = 2$) and Zhejiang ($n = 7$). It is interesting to see that 21 samples were carrying *tet*(X3) and *mcr-1* simultaneously (Table S3). Moreover, we found that 73 genes from the *tet*(X3)-bearing plasmid p34AB had close homologues (nucleic acid identities ranging from 95% to 100%) in the catalog of LPMs, account for 7.3% to 26.0% the total number of genes in the plasmid (Table S4). Although *tet*(X4) was discovered in *Enterobacteriaceae* bacteria (for example, *E. coli*) isolated from the stool sample in a pig farm and detected in food animals and humans, we did not identify this gene in the gut metagenomes ($n = 2931$). However, we found a gene with 98.7% amino acid sequence identity to *tet*(X4) was present in 90 chicken gut microbiomes of 495 samples from farms in China, which may be a potential tigecycline resistance gene.

In conclusion, our screening analysis for *mcr*, *tet*(X) and carbapenemases have substantially expanded our insights into the distribution of novel mobilized ARGs. In brief, the *mcr-9* gene, only detected in human gut samples, has been disseminating across at least three continents. *Tet*(X3), only identified in animals, has been widely spread in poultry samples collected from China LPMs. Due to limited genome data (metagenome), we are concerned that the worldwide distribution of these novel mobilized resistance genes *tet*(X) and *mcr* might be underestimated. Therefore, on the basis of our findings, we call for new epidemiological studies to evaluate the current intestinal carriage prevalence of potentially (oppor-

Table 1
Distribution of *tet*(X), *mcr* and carbapenemases as determined by retrospective analysis.

Origin	Country/Region	Positive samples (%)/ total samples							
		tet(X3)	*mcr-9*	*mcr-1*	*mcr-3*	*mcr-4*	*mcr-5*	NDM-1	NDM-5
Human	China	–	9 (2.4%)/368	27 (7.3%)/368	–	–	–	–	–
	America	–	1 (0.7%)/139	–	–	–	–	–	–
	Europe	–	12 (1.6%)/760	–	–	–	–	–	–
	UK	–	2 (0.8%)/250	–	–	–	–	–	–
	India	–	2 (1.8%)/110	–	–	–	–	–	1 (0.9%)/110
	Sweden	–	2 (2.0%)/98	–	–	–	–	–	–
Chicken at LPMs	China	22 (21.6%)/102	–	56 (54.9%)/102	1 (1.0%)/102	–	*b	*c	–
Duck at LPMs	China	8 (42.1%)/19	–	12 (63.2%)/19	3 (15.8%)/19	*a	*b	*c	–
Goose at LPMs	China	2 (33.3%)/6	–	3 (50.0%)/6	–	–	–	–	–
Pigeon at LPMs	China	1 (33.3%)/3	–	–	–	–	–	–	–
Chicken at farms	China	–	–	71 (14.3%)/495	–	–	–	–	40 (8.1%)/495
Pig	France, Denmark, China	–	–	–	–	–	–	–	–
Humans (total)	–	–	28 (1.4%)/2019	27 (1.3%)/2019	–	–	–	–	1 (0.05%)/2019
Animals (total)	–	33 (3.6%)/912	–	142 (15.6%)/912	4 (0.4%)/912	*a	*b	*c	40 (4.4%)/912

The dashes indicate that genes (full sequence, identity = 100%) have not been detected or are not applicable. *a, raw reads mapping identified *mcr-4* gene fragments in one poultry gut sample. *b, raw reads mapping identified *mcr-5* gene fragments in two poultry gut samples. *c, raw reads mapping identified NDM-1 gene fragments in five poultry gut samples.

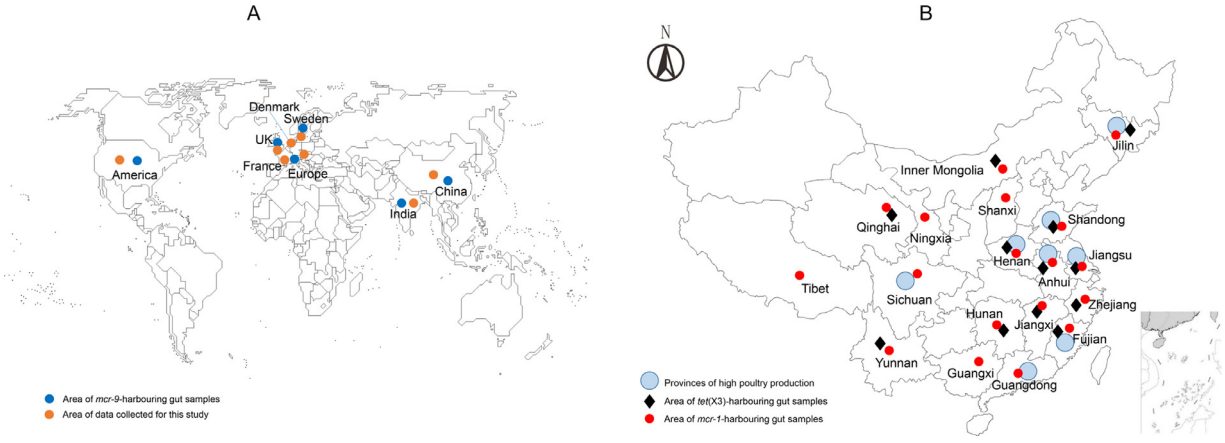

Fig. 1. A,B. Overview of the metagenome samples carrying the plasmid-mediated tigecycline resistance genes *tet*(X) and colistin resistance genes *mcr* around the world in this study. A, Distribution of novel *mcr-9* gene in the gut microbiome of human and animal across the world. B, Map of animal gut samples carrying *tet*(X3) and *mcr-1* gene in China.

tunistic) pathogenic bacteria carrying *mcr, tet*(X) and carbapenemases. Moreover, we need to urgently prevent the potential nightmare scenario (no effective antibiotics can be used to treat MDR infections) in the future, which deserves worldwide attention and strengthen related research immediately.

Declaration of Competing Interest

The authors declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2019.09.003.

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Analysis of colonization and infections during extracorporeal membrane oxygenation in children

We have read with interest the article of Schilcher et al.[1] on *Candida albicans* infection of the extracorporeal membrane oxygenation (ECMO). We are concerned about the role of *Candida* spp colonization in pediatric patients on ECMO. The prevalence of infection on ECMO is around 10–12%, according to data from the Extracorporeal Life Support Organization (ELSO),[2] and varies with age: for newborns, 4.4–8.4%; for pediatric, 15.3–19.7%, and for adults, 19.5–24%. The main risk factors for infections are the ECMO duration, the severity of the illness that prompted the ECMO run, and comorbid conditions.[3] ECMO shares important characteristics with cardiopulmonary bypass. The blood is drained to a circuit that acts as a mechanical heart and lungs, and then is returned to the patient through a vein or an artery. The blood is exposed to artificial materials and a systemic inflammatory response is activated with the release of pro-inflammatory cytokines.[4] In this context, differential infection diagnosis is difficult, especially in younger patients. Although there is not a recommendation from the ELSO to use antibiotic prophylaxis, a high percentage of centers use it.[5,6] The use of antifungal prophylaxis is less extended (2%), although most infections are caused by *Candida* spp.[2] Due to this, we analyzed the prevalence of colonization and infection in children during ECMO. All patients who were on ECMO in our Pediatric Intensive Care Unit (PICU) were included in this retrospective review from 2000 to 2019. All data related to infection was collected. The diagnosis of infection was based on the definitions from the Center for Disease Control.[7] The following were considered as positive colonization diagnoses: positive endotracheal cultures for fungal organisms ($<10^5$ colonies); fungi isolated in urine samples without clinical signs or alterations in the sediment; or a positive circuit surface culture ($<10^4$ colonies). Positive cultures isolated after ECMO were not included due to the difficulty in establishing a causative relationship with ECMO. Routine surveillance cultures (including blood, tracheal aspirate, urine, and circuit surface) were obtained every 48 h during the ECMO run. The study was approved by the Institutional Ethics Board.

Sixty-five patients were included. 53.8% (35) were males and the median age was 0.65 years (IQR 0.13–3.22). The cannulation was initially venoarterial in 48 patients (73.8%). The median number of days on ECMO was 6 (IQR 3–10), and the length of stay in the PICU was 23 (IQR 11.5–46). Forty-five (69.2%) survived.

Fifty-two patients (80%) received antibiotics before ECMO and a bacterial infection was confirmed in 21 (32.3%). Sixty-four patients (98.5%) received antibiotics during ECMO. The main combinations of antibiotics were vancomycin and amikacin (15/64, 23.4%), vancomycin and cefotaxime (14/64, 21.9%), and vancomycin and meropenem (12/64, 18.8%). The antibiotic-free days during ECMO were zero except for one patient with tracheal hypoplasia, placed on ECMO before surgery due to the impossibility of intubation.

Twelve patients (18.5%) were colonized during ECMO. The median number of days in PICU until colonization was 7.5 (IQR 3–8) and on ECMO was 3.5 (IQR 2–7). The colonization was detected in respiratory samples in 11 patients (91.7%) and in a urinary sample in one patient. Eleven patients (91.7%) were colonized by *Candida* spp (5 *parapsilosis*, 3 *albicans*, and 2 *tropicalis*). *Enterobacter cloacae* colonized one patient. Seven patients (10.8%) received antifungal prophylaxis.

Sixteen patients (24.6%) were infected during ECMO, eight of whom (50%) had been previously colonized during ECMO: the colonization and infection were by *Candida* in two, and the others were colonized by *Candida* and infected by bacteria. Table 1 shows the relationship between infected and non-infected patients. Table 2 compares bacterial and fungal infections. The bacteria isolated were *Stenotrophomonas maltophilia* (4, 25%), *Pseudomonas aeruginosa* (2, 12.5%), *Enterobacter cloacae,* and *Escherichia coli* (2 each, 12.5%). All the fungal infections were caused by *Candida* spp (2 *tropicalis*, 1 *glabrata,* and 2 *albicans*). There were 9 positive tracheal samples (all bacterial), 5 positive blood samples (3 bacterial, 2 fungal), and 6 positive urinary samples (2 bacterial, 4 fungal).

The Kaplan–Meier analysis is represented in Fig. 1. The colonization occurred before infection and the fungal infection occurred before the bacterial infection. In the stepwise logistic regression analysis, a longer duration of ECMO (>10 days, OR 5.225 [CI 1.265–21.588]), and colonization during ECMO (OR 10.007 [CI 2.213–45.245]) were independently associated with a higher risk for infections.

This is the first time that colonization in pediatric ECMO patients is being reported on, and it seems that colonization is related to a higher risk of infection during ECMO. Patients on ECMO are vulnerable due to their critical state and the invasive devices required, not only the circuit itself. In our institution, cultures were routinely collected and allowed the detection of colonization. According to our results, 50% of the patients who developed an infection during ECMO were previously colonized

Table 1
Comparison of baseline characteristics in patients with infectious and non-infectious complications during ECMO.

Variable	Non-Infection ($n = 49$)	Infection ($n = 16$)	p
Age (years)	0.9 (0.16–3.91)	0.27 (0.07–1.84)	
Male	26 (53.06)	9 (56.25)	0.824
CPR before ECMO	12 (24.5)	4 (25)	0.967
VA ECMO	39 (49.59)	9 (56.25)	0.065
VV-VA conversion	3 (6.12)	3 (18.75)	0.585
Need for RRT	23 (46.94)	7 (43.75)	0.772
Days in PICU until ECMO	2 (1–4)	2 (1–5)	0.697
Days in ECMO	5 (3–9)	10.5 (6.3–16.3)	0.001
Days in PICU	20 (7.5–40)	35 (23.3–49.3)	0.021
Days in hospital	31 (7.5–67)	35.5 (28.5–60.8)	0.304
Days in PICU until infection		8.5 (7.3–10.5)	
Days in ECMO until infection		6 (3.3–7)	
Days in PICU until colonization	8 (4.3–10.3)	6 (3–8)	0.368
Days in ECMO until colonization	5.5 (1.8–10.3)	3 (2–6.3)	0.461
Colonization	4 (8.16)	8 (50)	0.000
Antifungal prophylaxis	6 (12.24)	1 (6.25)	0.502
Bacterial infection before ECMO	20 (40.82)	1 (6.25)	0.014
Death	17 (34.69)	3 (18.75)	0.230

Categorical variables expressed as frequencies and percentages, compared with the chi-squared test. Continuous variables expressed as median and interquartile range, compared with the Mann–Whitney test. CPR = cardiopulmonary resuscitation; ECMO = extracorporeal membrane oxygenation; MV = mechanical ventilation; PICU = pediatric intensive care unit; VA = venoarterial; VV = venovenous.

Table 2
Comparison between bacterial and fungal infections.

Variable	Fungal infection ($n = 5$)	Bacterial infection ($n = 11$)	p
Age (years)	0.2 (0.9–5.7)	0.35 (0.05–1.9)	0.827
Male	2 (40)	7 (63.6)	0.377
VA ECMO	3 (60)	6 (54.5)	0.838
VV-VA conversion	2 (40)	1 (9.1)	0.142
Need for RRT	2 (40)	5 (45.5)	0.838
Days in PICU until ECMO	1 (20)	3 (1–5)	0.913
Days on ECMO	10 (6.5–22.5)	11 (6–17)	0.827
Days in PICU	24 (23–33)	37 (26–55)	0.145
Days in hospital	30 (26.5–35)	52 (28–66)	0.145
Days in PICU until infection	8 (4–11.5)	9 (8–9)	0.857
Days on ECMO until infection	4 (2–7)	6 (5–8)	0.857
Colonization	2 (40)	6 (54.5)	0.590
Antifungal prophylaxis	0 (0)	1 (9.1)	0.486
Bacterial infection before ECMO	1 (20)	0 (0)	0.126
Death	2 (40)	1 (9.1)	0.142

Categorical variables expressed as frequencies and percentages, compared with the chi-squared test. Continuous variables expressed as median and interquartile range, compared with the Mann–Whitney test. ECMO = extracorporeal membrane oxygenation; MV = mechanical ventilation; PICU = pediatric intensive care unit; VA = venoarterial; VV = venovenous.

(all by *Candida*). Serial cultures could allow for the detection of the most vulnerable patients that could develop an infection. ELSO does not recommend prophylactic antibiotics because they have not demonstrated their ability to reduce the risk of infection. Our aggressive antibiotic policy and the overuse of broad-spectrum antibiotics have conditioned our high rate of infection, although lower than others reported.[8] Our unit has not a high rate of resistance, but the trend is to use broad-spectrum antibiotics due to the critical state of patients. The infections were mostly respiratory and caused by bacteria. However, fungal infections were frequent and occurred before bacterial infections. In ECMO, biofilms play an important role in the development of fungal infection,[9] so a good option for prophylaxis and treatment will have the ability to penetrate biofilm, like micafungin.[10] The recommended dose for micafungin is currently 2.5 mg/kg/day for prophylaxis and 5 mg/kg/day for treatment. Due to the relationship between colonization and infection despite antibiotic prophylaxis, and the risk factors for fungal infections (invasive devices, broad-spectrum antibiotics…), the use of antifungal prophylaxis should be considered. The impact of this measure should be thoroughly analyzed in the future. ELSO recommends the "cautious, but aggressive" use of antifungal prophylaxis in high risk patients.[3]

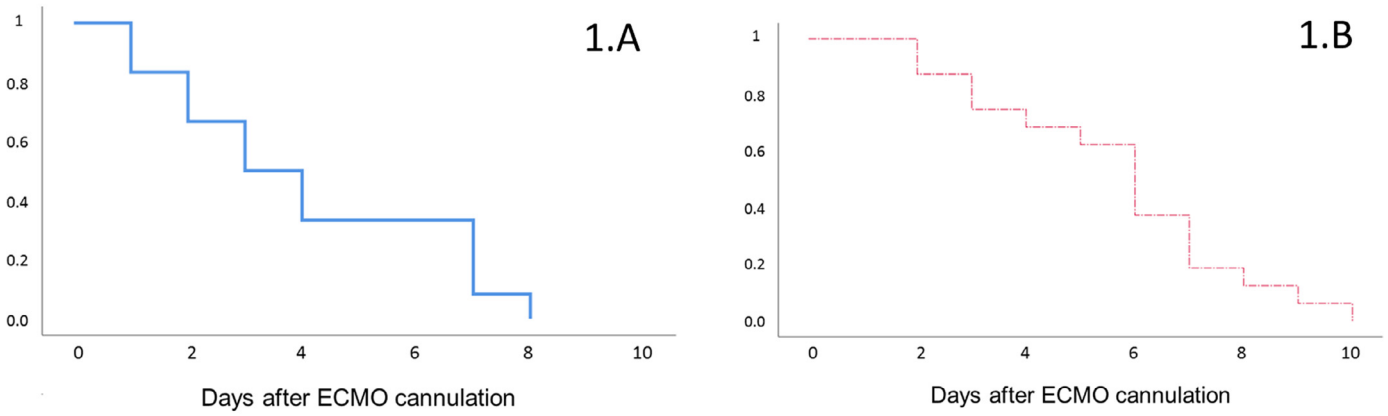
Financial discloser

The authors declare no relevant financial or nonfinancial relationships to disclose.

Declaration of Competing Interest

None.

Plot 1



Plot 2

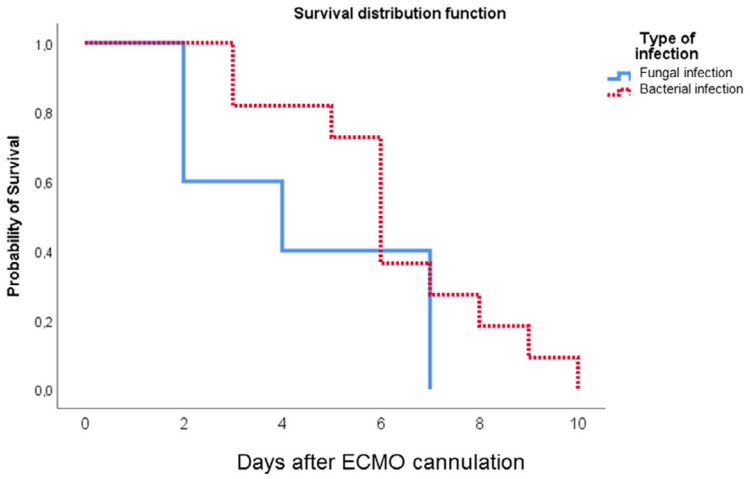


Fig. 1. Plot 1: Kaplan-Meier analysis of the time to event, considering the event as: the colonization (plot 1.A), infection (plot 1.B) and time as days on ECMO until the event. Plot 2: Kaplan-Meier analysis of the time to event (infection), based on the cause of the event (fungal or bacterial agent).

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Imported dengue fever and climatic variation are important determinants facilitating dengue epidemics in Southern Taiwan

Dear Editor,

We read with interest the recent research article by Wang et al. studying the epidemic characteristics of the 2014 dengue outbreak in Guangdong Province, China.[1] Dengue has recently emerged or re-emerged in many countries worldwide with the number of cases and the range of the virus increasing annually. Taiwan has experienced two large dengue outbreaks in the recent decade with high morbidity and mortality noted. Reports from Taiwan Center for Disease Control (Taiwan CDC) indicated that the major dengue epidemics occurred in the south of Taiwan, mainly in the two southern cities of Tainan and Kaohsiung.[2] The reasons regarding the severe dengue epidemics occurrence remain unclear.

Dengue fever (DF) is an arthropod-borne disease caused by dengue virus (DENV). DENV is transmitted by *Aedes* mosquitoes, mainly *Aedes aegypti* and *Aedes albopictus*. DENV is a *Flavivirus* belonging to the *Flaviviridae* family. DENV comprises of four antigenically distinct serotypes. According to the World Health Organization (WHO) reports, around 2–3 billion people are living in dengue epidemic areas.[3,4] The incidence of dengue fever has increased more than 30 folds during past decades.[5,6]

Taiwan is an island, located in the western Pacific Ocean, neighbored by countries such as China, Japan and Philippines. The south of Taiwan is located in tropical area, providing an ideal environment for the *Aedes* mosquitos, especially *Aedes aegypti.* The abundance of *Aedes aegypti* has shown correlation with dengue epidemics in Taiwan.[7] Taiwan has experienced intermittent dengue epidemics with intervals of several years to decades. In the recent decade, Taiwan has experienced a large dengue outbreak in 2014, with a total of 15,732 DF cases reported.[8] In 2015, there was a more severe DF outbreak affecting two southern cities of Taiwan. A total of 70,341 dengue fever suspected cases were reported, among them, 43,784 were laboratory confirmed DENV cases.[2] Previous studies report that imported DF and climatic changes are risk factors correlated with dengue outbreaks.[9,10] Accordingly, we conducted an epidemiological survey to dissect the factors correlated with the dengue epidemics in these two southern cities of Taiwan, mainly focused on the impact from dengue imported cases and climatic variations.

The climatic data including temperature, humidity and precipitation were collected from Taiwan Central Weather Bureau. The DF information was offered by Taiwan CDC and Kaohsiung Medical University Hospital. Dengue epidemic and non-epidemic years were classified according to the criteria determined previously.[8] The study was approved by the Institutional Ethics Committee of the Kaohsiung Medical University, Taiwan. The lagged-time Poisson univariate and multivariate regression analyses were performed and the detail were described in supplements.

The correlation and pattern of climatic factors and imported DF cases with dengue outbreaks in Taiwan are illustrated (Supplementary Fig. 1). Results from time-lag univariate regression analysis in dengue epidemic years indicate that the increase of humidity, precipitation and imported DF cases significantly correlated with dengue incidences at 1 month lag ($p < 0.001$), 2 month lag ($p < 0.05$) and 1-to-3 month lags ($p < 0.05$), respectively, in Kaohsiung city. No significant correlation was found among these climatic factors as well as imported DF cases with dengue occurrence in Tainan city ($p > 0.05$) (Table 1).

Time-lag multivariate regression analyses indicates that changes of temperature, humidity and precipitation significantly correlated with dengue incidences at 1-to-3 month lags, 1 month lag and 3 month lag, respectively, in Kaohsiung city ($p < 0.001$) (Table 1). DF imported cases were significantly correlated with dengue incidences at 1-to-2 month lags during dengue epidemic years in Kaohsiung city ($p < 0.001$). When adjusting with imported DF, more significant correlation of these climatic factors with dengue incidences in Kaohsiung city was observed. The changes of temperature, humidity and precipitation significantly correlated with dengue incidences at 3 month lag, 1-to-3 month lags and 1 month lag, respectively ($p < 0.001$). However, no significant correlation was seen among these climatic factors as well as imported DF cases with dengue occurrence in Tainan city ($p > 0.05$) (Table 1).

In non-dengue epidemic years, climate factors and DF imported cases were not significantly correlated with dengue incidences in either Kaohsiung or Tainan city ($p > 0.05$). Of note, temperature and humidity significantly correlated with dengue incidences at 2 month lag in Tainan ($p = 0.0032$) and Kaohsiung city ($p = 0.0396$) under univariate and multivariate time-lag analyses, respectively (Table 2).

In this study, we demonstrate the impact of the variations of temperature, humidity and precipitation on DF occurrence in Kaohsiung city during dengue epidemic years. These weather changes are beneficial to DENVs dissemination and imported cases might be an initiating factor subsequently triggering occurrence of dengue epidemics in Kaohsiung city. However, in Tainan city,

Table 1
Time-lagging analysis of the correlation of climatic factors and imported DF cases with the incidence of DENVs infected cases during dengue epidemic years in Taiwan.

			Temperature		Humidity		Precipitation		Imported DF	
City	Model	Time lag	Beta	*p* Value	Beta	*p* Value	Beta	*p* Value	beta	*p* value
Kaohsiung	Single factor	Lag 1 month	0.215	0.1535	1.165	0.0002	0.0070	0.2549	0.022	0.0344
		Lag 2 month	−0.122	0.4615	5.198	0.1829	0.1138	0.0296	0.043	0.0000
		Lag 3 month	0.204	0.2200	7.608	0.8861	0.9868	0.0664	0.030	0.0072
	Multiple factors	Lag 1 month	0.407	0.0179	0.847	0.0049	−0.0058	0.3082	–	–
	w/o imported	Lag 2 month	−0.455	0.0067	3.708	0.3709	−0.0013	0.9780	–	–
		Lag 3 month	0.801	0.0008	97.221	0.1490	1.8298	0.0006	–	–
	Multiple factors	Lag 1 month	0.013	0.9310	1.033	0.0000	−0.0169	0.0002	0.030	0.0000
		Lag 2 month	0.174	0.3118	10.192	0.0040	−0.0322	0.3553	0.031	0.0000
		Lag 3 month	0.805	0.0000	179.12	0.0004	0.0337	0.9478	0.005	0.4155
Tainan	Single factor	Lag 1 month	−0.569	0.1137	−0.704	0.7431	0.0081	0.7808	−0.047	0.3381
		Lag 2 month	0.366	0.3037	−15.777	0.3578	−0.2212	0.3588	−0.037	0.5336
		Lag 3 month	0.222	0.5203	−298.87	0.0850	−3.0825	0.1213	−0.040	0.6625
	Multiple factors	Lag 1 month	−0.789	0.1628	0.041	0.7943	0.0025	0.4636		
	w/o imported	Lag 2 month	−0.303	0.5861	−0.562	0.6608	0.0227	0.5005		
		Lag 3 month	−0.019	0.9783	2.093	0.8918	0.0259	0.9341		
	Multiple factors	Lag 1 month	−0.682	0.1471	0.594	0.5758	−0.0241	0.5325	0.044	0.2057
		Lag 2 month	0.656	0.2585	3.212	0.8107	−0.0884	0.7583	0.014	0.6519
		Lag 3 month	0.623	0.2330	−23.943	0.9169.	0.7064	0.7939	−0.030	0.2603

The dengue epidemic years (moderate-to-large DF outbreak) were identified and referenced by previous studies. The dengue epidemic years were categorized as 2002, 2006, 2010, 2011, 2014 and 2015. Models were adjusted for year and month.

Table 2
Time-lagging analysis of the correlation of climatic factors and imported DF cases with the incidence of DENVs infected cases during dengue non-epidemic years in Taiwan.

			Temperature		Humidity		Precipitation		Imported DF	
City	Model	Time lag	Beta	*p* Value	Beta	*p* Value	Beta	*p* Value	Beta	*p* Value
Kaohsiung	Single factor	Lag 1 month	−0.212	0.1950	0.485	0.1125	0.0057	0.2760	0.006	0.7162
		Lag 2 month	−0.049	0.7542	−5.270	0.0945	0.0228	0.5729	−0.024	0.1859
		Lag 3 month	0.089	0.5903	12.480	0.6734	0.2911	0.3743	−0.016	0.3842
	Multiple factors	Lag 1 month	−0.180	0.3472	0.503	0.1947	0.0003	0.9592	–	–
	w/o imported	Lag 2 month	−0.099	0.5678	−7.615	0.0591	0.0361	0.4455	–	–
		Lag 3 month	0.132	0.4635	6.833	0.8704	0.2374	0.5998	–	–
	Multiple factors	Lag 1 month	−0.198	0.3455	0.392	0.3917	0.0003	0.9703	0.017	0.4417
		Lag 2 month	−0.101	0.5871	−9.490	0.0396	0.0365	0.5264	−0.019	0.3423
		Lag 3 month	0.142	0.4446	11.927	0.8023	0.1673	0.7337	−0.027	0.1346
Tainan	Single factor	Lag 1 month	−0.134	0.5931	0.482	0.2604	−0.0087	0.2054	0.028	0.1140
		Lag 2 month	−0.754	0.0032	4.526	0.3021	0.0258	0.6028	0.008	0.7237
		Lag 3 month	−0.335	0.1186	15.759	0.7400	1.0459	0.0652	−0.066	0.0634
	Multiple factors	Lag 1 month	−0.134	0.6478	0.562	0.2361	0.0046	0.6910	–	–
	w/o imported	Lag 2 month	−0.630	0.0508	3.014	0.5246	−0.0213	0.8146	–	–
		Lag 3 month	−0.264	0.3207	19.968	0.7305	0.0536	0.9479	–	–
	Multiple factors	Lag 1 month	−0.107	0.7373	0.184	0.7813	0.0073	0.5585	0.006	0.8832
		Lag 2 month	−0.514	0.1588	3.890	0.5634	−0.0372	0.7288	−0.007	0.8078
		Lag 3 month	−0.279	0.4368	23.422	0.7124	−0.1617	0.8591	−0.015	0.7269

The dengue non-epidemic years were identified and referenced by previous studies. The dengue non-epidemic years were categorized as 2001, 2003, 2004, 2005, 2007, 2008, 2009, 2012 and 2013. Models were adjusted for year and month.

these climatic changes did not favor DENV transmission, even though imported DF appeared in Tainan city. These results point to Kaohsiung city as an epicenter of dengue in Taiwan. Therefore, the environmental changes correlated with DF epidemics should be strictly monitored. Previous reports support our findings indicate that most dengue epidemics occurred in Kaohsiung city,[9] imported dengue was the major source prompting dengue outbreaks in Taiwan[10] and climate changes are important factors influencing the incidence and distribution of dengue.[6] Further, changes of humidity correlated with DF incidences in non-dengue epidemic years in Kaohsiung city thus, humidity is an essential factor in maintaining those sporadic DF cases in Kaohsiung city. Regarding Tainan city, only temperature is correlated with DF incidences, suggesting that the variations of temperature may be associated with *Ades mosquitoes*' activities subsequently having the impacts on dissemination of DENVs. Further investigations are still needed to address these correlations. In addition, some of the coefficient estimates in the regression model were very large, especially for lagged effect of humidity. This may result from the smaller DF number of months. Data of more years of observations in the future may be needed for more stable estimates.

Finally, this study reveals that the incidence of DF in Kaohsiung city was associated with weather factors and imported DF. Continuous surveillance of imported and indigenous DF cases as well as implantation of environmental and vector control plans are urgent demand to control dengue outbreaks in Taiwan.

Declaration of Competing Interest

No potential conflict of interest was reported by the authors.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2019.08.010.

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The recombination hot spots and genetic diversity of the genomes of African swine fever viruses

Dear Editor,

Recently, the phylogeographic patterns of African swine fever virus (ASFV) is studied in this journal.[1] ASF possess a devastating threat to pig agriculture. Endemic to South and East Africa, ASFV has jumped out of Africa several times, with the most recent event being its introduction to Georgia in 2007 where it then spread through Russia and Eastern Europe. In August 2018, China reported its first ASF case in northeastern China.[2] Since then, it has quickly swept through China, and crossed borders to Vietnam, Cambodia, North Korea, and Mongolia. Effective preventive and biosafety measures can have a significant impact on limiting the spread of ASFV, but the low biosafety level of backyard farms, and weak veterinary infrastructure makes the eradication of this virus difficult. The long-term persistence of ASF in these countries, increases the risk of the spread of ASFV to other Southeast Asian countries and makes it imperative to improve our knowledge about this pathogen. Genotypes of ASFVs are based on its p72 gene, however, the p54 gene shows much higher diversity than p72.[1] In addition, the phylogenetic positions of some viruses based on p54 is inconsistent with that deduced from p72,[3] suggesting that recombination within this virus may occur. To examine this question, in this study, we collected all available ASFV genomes to investigate their genetic diversity and test for potential recombination events.

All 42 available ASFV genomes were collected from GenBank (Table S1), which were then aligned using MAFFT software, a fast multiple sequence alignment program.[4] Alignment of these genomes showed that regions at the left and right ends of the genome were highly variable, containing many insertions or deletions, and thus a robust alignment of these regions could not be obtained. Therefore, for the following analyses we excluded the beginning and the end of the genomes. When the R8 (accession number MH025916.1) ASFV genome was set as the standard genome, aligned fragments, which were used in the following analysis, ranged from the 22-R to the MGF 360-21R genes. Nucleotide diversity (π) was calculated across the genome in 1 kb sliding windows with a step size of 200 bp, and shows great variation in diversity (0.0151–0.2069) in the different regions (Fig. 1A). The X64R, EP152R, EP153R, EP402R, EP364R and CP2475L genes are located in regions with very high genetic diversity, while the P72 and P54 genes are located in regions with very low genetic diversity. Mutations generated during replication that have a better ability to replicate or be transmitted within the

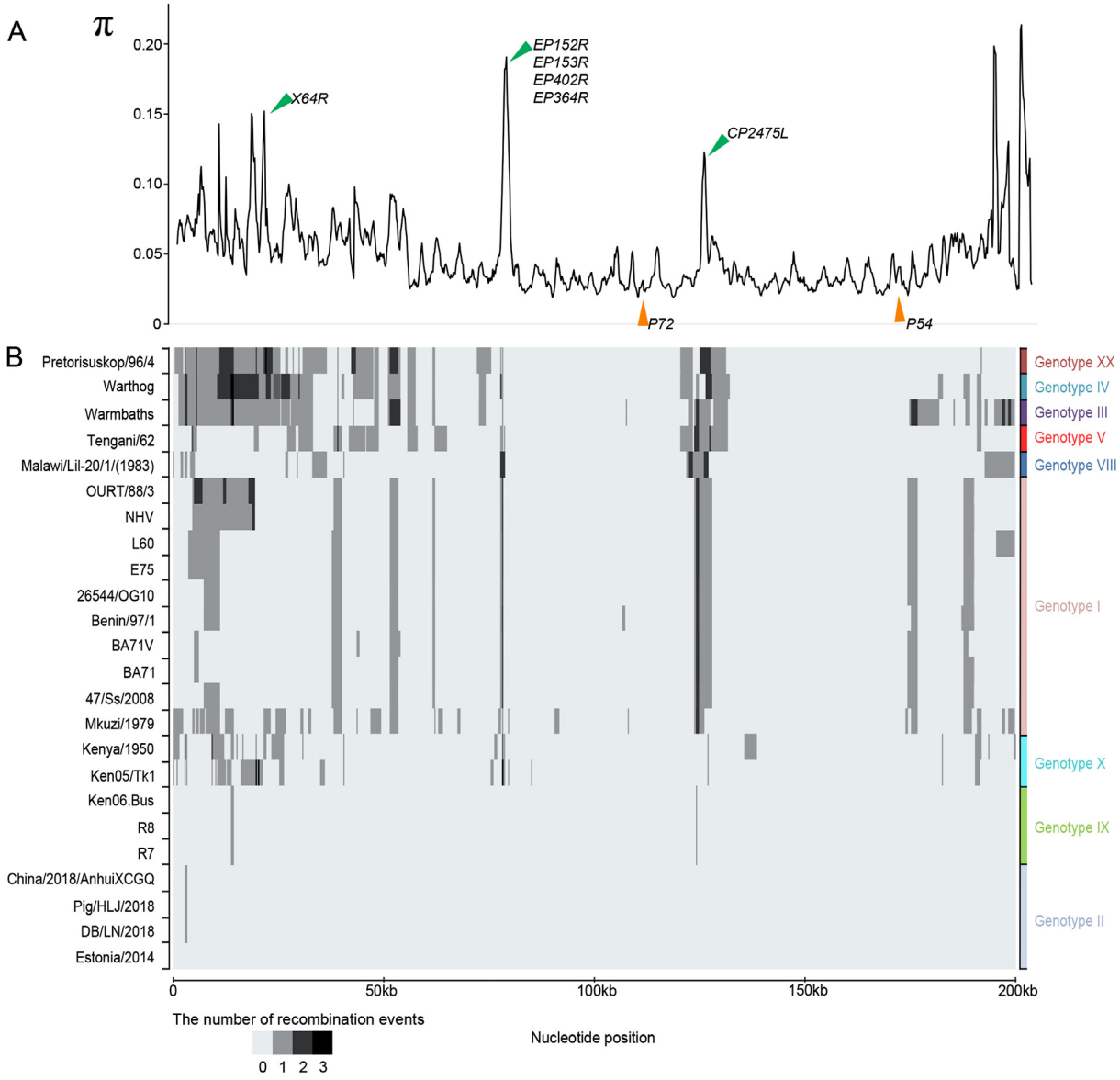

Fig. 1. Genomic diversity and recombination in ASFV genomes. (A) Nucleotide diversity (π) was calculated in 1 kb sliding windows with a step size of 200 bp. Genes located in regions with high nucleotide diversity are marked in green, while the two most commonly studied genes, p54 and p72, are marked in orange. (B) Summary of recombination events in the available ASFV genomes identified by RDP4. Recombination regions were marked in black.

host, or can avoid the immune response of the host, have selective advantage, in terms of viral fitness, and may become fixed in a viral population. Gene regions that are associated with antigenic drift may have a higher genetic diversity as they allow the viruses to escape the host immune system. Genomic regions with low levels of genetic diversity are likely subjected to strong purifying selection and encode conserved functions necessary for viral replication or packaging. Therefore, gene regions with abnormally high levels of genetic diversity are valuable candidates for functional experiments.

Our study revealed that the ASFV genomes contain some regions with high genetic diversity (Fig. 1A). Random mutation across the genome cannot explain this result, therefore, we tested whether recombination events caused this phenomenon. Recombination analysis was performed by the RDP4 program using the 3seq, bootscan, chimaera, genecov, lard, maxchi, rdp and siscan detection methods.[4] We considered a recombination event to be reliable only if it was detected as a significant signal by at least three different methods. From the 42 aligned ASFV genomes, we found reliable evidence for 152 recombination events (Table S2).

The region around the CP2475L gene (from CP123L to CP312R genes) of the X genotype had only one recombination event. We use genotype X as an example to show recombination in detail. Similarity plots and bootscanning analyses were also used for recombination detection, which revealed that the recombinant region of the CP2475L gene of the genotype X is from 128,471 to 129,086 bp (Fig. 2). This result overlaps with the recombination region (128,458–129,208 bp) calculated by RDP (Table S2). Phylogenetic analysis of the recombinant region indicated that genotypes X and V cluster together, while phylogenetic tree generated for other regions of the genomes showed that genotype X clusters with genotype IX (Fig. 2C). Phylogenetic analysis supported the conclusion that genotype X is a recombinant virus that acquired a part of its genome from a virus of genotype V on a genomic background of genotype IX. Recombination is a well-known important source of genetic variability in viruses. Our study of ASFV genomes

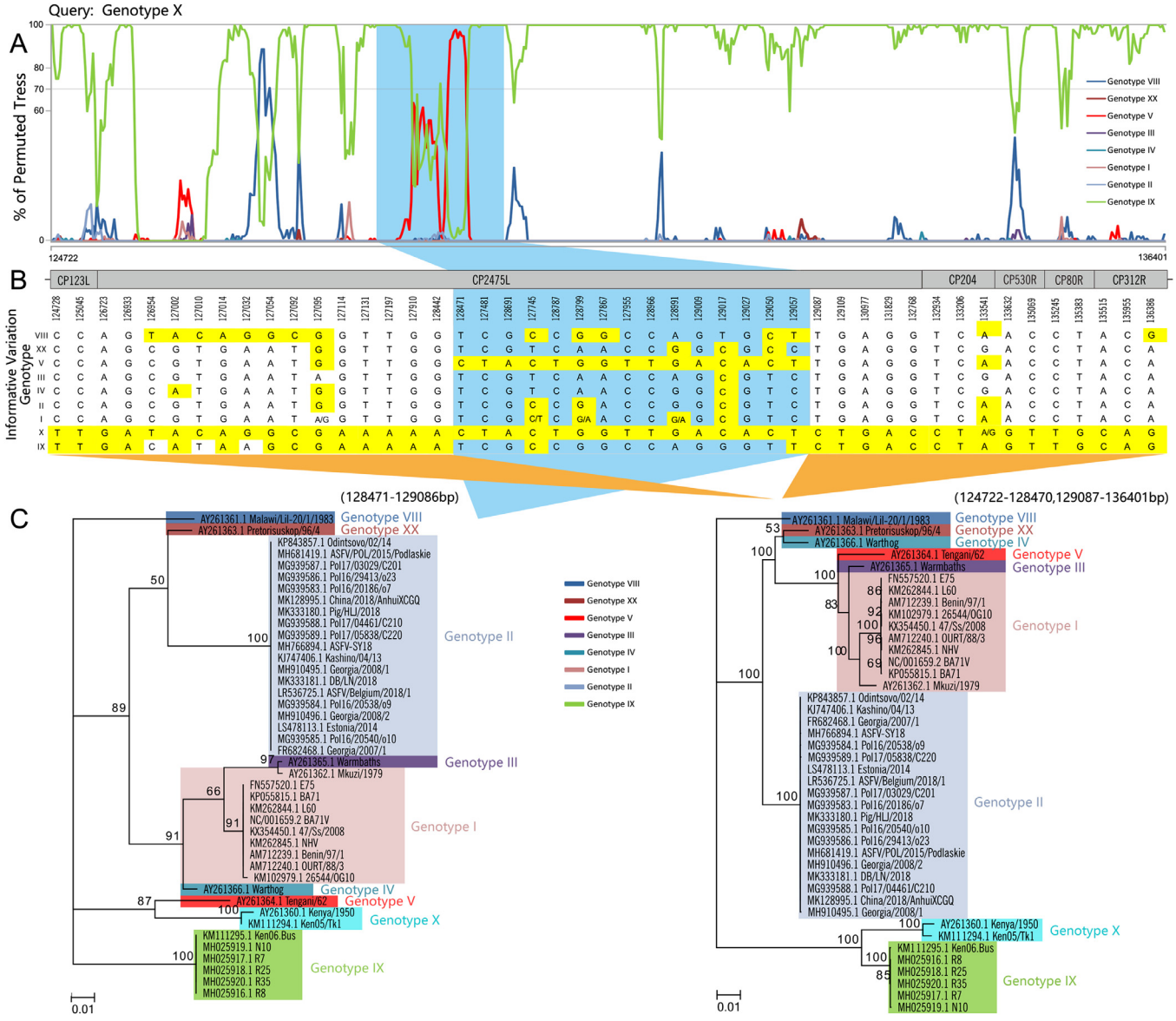

Fig. 2. Recombination analysis of Genotype X. (A) Bootscanning recombination analysis of genotype X based on variable genomic sites. Dashed horizontal line indicates 70% bootstrap support. (B) Variable sites from the CP123L to the CP312R gene region, with the recombination breakpoint within the CP2475L gene marked in blue. (C) Maximum-likelihood phylogenetic trees inferred for the outer (left) recombinant region and inner (right) non-recombinant regions, indicating that the genotype X is a recombinant virus that acquired a part of CP2475L from genotype V while the remaining genomic regions are from genotype IX.

has revealed that recombination hotspots have greatly raised their genetic diversity (Fig. 1). Thus, it is likely that recombination facilitates ASFV effectively generating diverse genetic strains to aid in the evasion of host immunity, and increase its virulence and pathogenicity.[5]

Previous work has shown that live attenuated viruses have poor cross-protection against heterologous challenges.[6] Thus, a concern with the use of ASFV vaccines is the genetic diversity of circulating strains. However, in the context of cross-protective immunity what is a heterologous ASFV strain? The ASFV genome encodes 150–200 proteins.[7] Current ASFV genotyping relies predominantly on the p72 gene, however, p72 located in a very low genetic diversity region (Fig. 1A), thus, this gene provides very limited insight on the genetic diversity of ASFV. p72-based ASFV genotypes do not fully correlate with available cross-protection data and may also be of limited value in predicting vaccine efficacy.[8] Knowledge of ASFV strain diversity, and the antigenic diversity of the relevant protective antigens, is critical for the successful design of ASFV vaccines, and for development of rapid diagnostic methods capable of discriminating among viruses and, thus, predicting the potential efficacy of a given vaccine against a ASFV field isolate.[9]

In endemic areas (South and East Africa), it is difficult to eliminate the natural reservoir of this virus in warthogs and soft tick vectors. In Europe, the Caucasus region and Russia, wild boars, which are as susceptible to ASF as pigs, are its primary reservoir.[10] Wild boars, which can easily move across national borders, are widely distributed across Eurasia. In addition, in Southeast Asia, the low biosafety of backyard farms, weak veterinary infrastructure and limited funds will likely make the eradication of ASF based on early diagnostic detection, culling of ASF-positive animals and strict sanitary measures nearly impossible, and thus, the emergence and re-emergence of ASF will pose an increasing threat to global pig agriculture. Vaccination is one of the most valuable defensive tools. Development of an effective vaccine that can cross-protect against infections by several genotypes is urgently needed. However, to date, no vaccine has become available due to a

number of key factors, including the lack of identification of protective antigens, incomplete understanding of virus–host cell interactions and inadequate knowledge related to the diversity of viral strains currently circulating in the natural reservoirs.[9] Our study revealed that the wildly used p72 genotypes reflect limited information of the genetic diversity of ASFVs. The genetic diversity of different genomic regions of this virus vary greatly, with p72 being one of the regions with lowest diversity. In addition, we found evidence for frequent recombination within ASFV genomes. If live attenuated viruses are to be used as vaccines, then we need to be aware of their potential recombination with field strains.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary materials

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Utility of FilmArray® ME panel for prompt *Neisseria meningitidis* detection in non-cerebrospinal fluid samples– A case report

Dear Editor,

González-Donapetry and colleagues, in this Journal recently reported the limitations in the use of a multiplex PCR system in CNS infection.[1] *Neisseria meningitidis* is a leading cause of invasive infections associated with high mortality and morbidity, notably meningitis and septicemia.[2] Although a rare endemic disease in most countries, the epidemiology of meningococcal disease varies widely over time and in different geographic regions, with both hyperendemic and epidemic disease patterns occurring. Onset of disease in susceptible individuals may be very rapid, within hours, and the case fatality rate is high, especially among those presenting with septic shock, despite access to modern critical care. Individual susceptibility involves a complex relationship among environmental, host and bacterial factors, and prevention of meningococcal disease through behavior modification (e.g., avoiding tobacco smoke) and vaccination offers the best prospect for control.[3]

Etiological rapid diagnosis is key for the preventive management and therapeutic of invasive meningococcal disease. However, conventional methods for diagnosis are time-consuming and hampered by the difficulties in culturing the isolates from clinical specimens, especially due to early antibiotic treatment.[4] Therefore, sensitive, specific and rapid non-culture-based methods are valuable for early diagnosis, effective therapy, and prevention.

The FilmArray® ME Panel (BioFire Diagnostics LLC, Salt Lake City, UT, USA) is an in vitro diagnostic multiplexed PCR test for the simultaneous detection and identification of up to 14 bacterial, viral, and yeast pathogens directly from cerebrospinal fluid (CSF). These pathogens include: *Escherichia coli K1, Haemophilus influenzae, Listeria monocytogenes, Neisseria meningitidis, Streptococcus pneumoniae, Streptococcus agalactiae*, cytomegalovirus, enterovirus, herpes simplex virus 1 and 2, human herpesvirus 6, human parechovirus, varicella-zoster virus, and *Cryptococcus neoformans/gattii.* We describe the use of this technology, outside of its licensed use, to determine the causative agent of meningococcal sepsis in whole blood and skin biopsy samples.

A three-year-old boy was urgently transferred from a secondary hospital to our center, with the fundamental diagnosis of

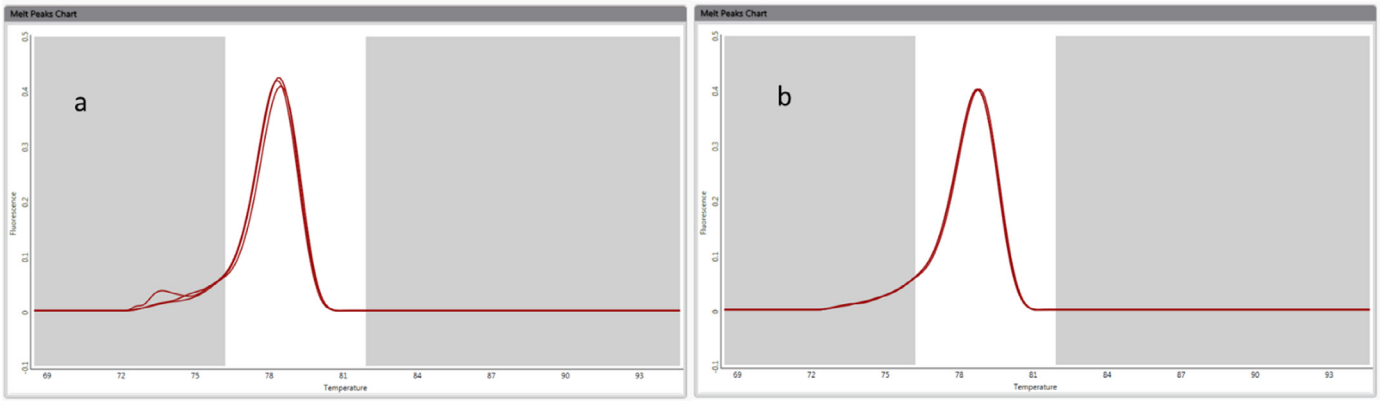

Fig. 1. Melting positive curves of the skin biopsy (a) and the whole blood sample (b) for *Neisseria meningitidis*.

catecholamine-refractory septic shock. His-parents reported a clinical picture of fever and decay of less than 24 h of evolution, as well as the appearance of purpuric lesions in the last hours. Meningococcal sepsis was then suspected and a first dose of iv ceftriaxone (50 mg/kg) was administered before transfer.

Upon arrival at our center, the patient was admitted to the Pediatric Intensive Care Unit (PICU) in poor general condition. High-dose iv cefotaxime (300 mg/kg/day) was prescribed due to high clinical suspicion of meningococcemia. Cerebrospinal fluid (CSF) could not be obtained for molecular analysis and culture due to patient instability. For this reason, blood cultures, whole blood sample and skin biopsy were obtained. Blood cultures and skin biopsy were cultured and, due to the need for a rapid diagnosis, we also analyzed them using the FilmArray® ME Panel assay. As this technique is only validated for CSF samples, our non-CSF samples were processed as follows; in the case of whole blood sample, we use the same volume of sample required for CSF (200 µl). In the case of the skin biopsy sample, it was first homogenized with thioglycolate broth (following Clinical Microbiology Departments Institutional protocols) and then, the FilmArray® ME Panel was processed with the same volume of the sample (200 µl). Both samples reported a positive result for *Neisseria meningitidis* (Fig. 1).

Afterward, whole blood sample was sent to the Spanish National Centre for Microbiology (Majadahonda, Madrid, Spain) for further characterization. *Neisseria meningitidis* serogroup C was confirmed.

Neisseria meningitidis is still a cause for concern among society in general and especially among health workers because its early diagnosis and treatment are critical to avoid life threating conditions and sequelae.[5] To improve clinical management and take epidemiological decisions (as prophylaxis to close contacts), prompt etiological diagnosis is vital.[6]

The classical microbiology methods used for the diagnosis of bacterial meningitis are based on the direct examination of cerebrospinal fluid Gram stain, culture and antigenic detection by agglutination of latex particles or conglutination.[7,8] Previous exposure to antibiotics, low CSF bacterial load and inadequate collection, transport and processing of the samples decrease the sensitivity of these methodologies.[8–10] The detection of infectious agents by the polymerase chain reaction (PCR), has acquired great importance during the last years for the diagnosis of bacterial meningitis increasing sensitivity and specificity compared to classical methods.[8,11]

In our case, the most important clinical limitations were that lumbar puncture could not be performed due to severe patient instability and culture-based tests for etiological diagnosis were not reliable due to prior exposure to antibiotics. For this reason, and aiming a rapid identification of the etiological agent, we successfully performed the FilmArray® ME Panel assay using whole blood and skin biopsy samples, yielding reliable identification of *Neisseria meningitidis*. Successful applications of this technique using alternative non-CSF samples (e.g., synovial fluid) have also been published recently.[4,12]

In conclusion, we report the successful use of the FilmArray® ME Panel technique using non-CSF samples for the prompt diagnosis of *Neisseria meningitidis* in a child diagnosed with refractory septic shock. As this could be outstandingly useful in patients in whom meningococcal meningitis or septicaemia are suspected but CSF sample cannot be obtained, more studies may be conducted to confirm these findings.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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