

Appendix 1

Details of the microbiological cultures

All the patients underwent blood culture by peripheral venous puncture at least once during fever after admission. Blood samples (8–10 mL) were injected into aerobic, anaerobic, and fungal blood culture bottles (BD BACTECTM, Becton, Dickinson, and Co., Franklin Lakes, NJ, USA) and then loaded into an automated continuous monitoring system (BD BACTECTM, USA) for 7 days. Other specimens such as pus, pleural fluid, peritoneal fluid, bile, cerebrospinal fluid, urine and sputum were cultured onto blood agar plates, chocolate agar plates, and fungal chromogenic plates for 7 days. If culture showed bacterial or fungal growth, strain identification was conducted by VITEK MALDI-TOF mass spectrometry (bioMérieux, Craponne, France). Antimicrobial susceptibility tests were conducted using Vitek 2 (bioMérieux, France).

Table S1 Demographic and clinical characteristics of the study participants

Characteristics	Noninfectious fever (n=11)	BSI (n=69)
Age (years)	60.6±12.3	59.4±15.8
Gender, male	7 (63.6)	50 (72.5)
Underlying infectious syndromes		
Abdominal infections	NA	26 (37.7)
Infective endocarditis ^a	NA	9 (13.0)
Catheter/device related infections ^b	NA	8 (11.6)
Urinary tract infections	NA	7 (10.1)
Skin and soft tissue infections	NA	7 (10.1)
Respiratory infections	NA	6 (8.7)
Infectious aneurysm	NA	2 (2.9)
Osteoarticular infections	NA	1 (1.4)
Septic thrombophlebitis	NA	1 (1.4)
Central nervous system infections	NA	1 (1.4)
Other/unknown infections	NA	1 (1.4)
Underlying risks		
Active malignancy	5 (45.5)	13 (18.8)
Diabetes mellitus	1 (9.1)	12 (17.4)
Long-term use of immunosuppressive therapy	0	7 (10.1)
Nosocomial infection	NA	19 (27.5)
Antibiotics within 4 weeks before admission	10 (90.9)	59 (85.5)
Duration of fever on admission (days)	17 [14–30]	20 [7–30]
Laboratory		
WBC (10 ⁹ /L)	10.4 [6.3–13.2]	8.9 [6.4–12.5]
ESR (mm/h)	80 [40–120]	41 [18–65]
CRP (mg/L)	136 [87–300]	94 [49.5–184.2]
PCT (ng/mL)	0.6 [0.1–1.1]	0.5 [0.2–3.3]

Data are expressed as mean ± standard deviation, n (%) or median [IQR]. ^a, including native valve endocarditis, prosthetic valve endocarditis and pacemaker-related endocarditis; ^b, including infections related to central line catheters, artificial blood vessels and stent implantation. BSI, bloodstream infection; WBC, white blood cell count; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; PCT, procalcitonin; NA, not applicable; IQR, interquartile range.

Table S2 Results of blood culture, blood mNGS and blood tNGS of the 80 patients enrolled in this study

Patient number	Blood culture	Blood mNGS	Blood tNGS	Underlying infectious syndromes
Patients with noninfectious fever (n=11)				
P1	Negative	Negative	Negative	NA
P2	Negative	<i>Streptococcus mitis</i>	Negative	NA
P3	Negative	Negative	Negative	NA
P4	Negative	Negative	Negative	NA
P5	Negative	Negative	Negative	NA
P6	Negative	<i>Haemophilus parainfluenzae</i>	Negative	NA
P7	Negative	Negative	Negative	NA
P8	Negative	Negative	Negative	NA
P9	Negative	Negative	Negative	NA
P10	Negative	Negative	Negative	NA
P11	Negative	Negative	Negative	NA
Patients with BSI (n=69)				
P12	<i>Streptococcus oralis</i> (<i>S. oralis</i>)	<i>S. oralis</i>	<i>S. oralis</i>	Infective endocarditis ^a
P13	<i>Staphylococcus aureus</i> (<i>S. aureus</i>)	<i>S. aureus</i>	<i>S. aureus</i>	Infective endocarditis
P14	<i>Escherichia coli</i> (<i>E. coli</i>)	Negative	<i>E. coli</i> , <i>Enterococcus faecium</i>	Abdominal infections
P15	Negative	<i>Treponema denticola</i> (<i>T. denticola</i>), <i>Fusobacterium nucleatum</i> (<i>F. nucleatum</i>)	<i>T. denticola</i> , <i>F. nucleatum</i>	Catheter/device related infections ^b
P16	Negative	<i>Klebsiella pneumoniae</i> (<i>K. pneumoniae</i>)	<i>K. pneumoniae</i>	Abdominal infections
P17	Negative	Negative	Negative	Abdominal infections
P18	Negative	Negative	<i>K. pneumoniae</i>	Abdominal infections
P19	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	<i>K. pneumoniae</i> , <i>S. aureus</i>	Infective endocarditis
P20	Negative	Negative	Negative	Abdominal infections
P21	Negative	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	Abdominal infections
P22	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>	Urinary tract infections
P23	Negative	Negative	<i>S. aureus</i>	Skin and soft tissue infections
P24	Negative	<i>Aeromonas veronii</i> (<i>A. veronii</i>)	<i>A. veronii</i>	Skin and soft tissue infections
P25	Negative	Negative	<i>K. pneumoniae</i>	Abdominal infections
P26	Negative	<i>Vibrio vulnificus</i> (<i>V. vulnificus</i>)	<i>V. vulnificus</i>	Skin and soft tissue infections
P27	Negative	Negative	<i>E. coli</i>	Urinary tract infections
P28	Negative	<i>K. pneumoniae</i> , <i>Lactobacillus iners</i>	<i>K. pneumoniae</i>	Abdominal infections
P29	Negative	Negative	<i>E. coli</i>	Urinary tract infections

Table S2 (Continued)

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Patient number	Blood culture	Blood mNGS	Blood tNGS	Underlying infectious syndromes
P30	Negative	<i>Enterococcus raffinosus</i> (<i>E. raffinosus</i>)	<i>E. raffinosus</i> , <i>K. pneumoniae</i>	Abdominal infections
P31	Negative	Negative	<i>K. pneumoniae</i>	Catheter/device related infections
P32	Negative	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	Abdominal infections
P33	<i>Streptococcus sanguinis</i> (<i>S. sanguinis</i>)	<i>S. sanguinis</i>	<i>S. sanguinis</i>	Infective endocarditis
P34	Negative	<i>E. coli</i>	<i>E. coli</i>	Abdominal infections
P35	Negative	<i>E. coli</i>	<i>E. coli</i> , <i>Candida parapsilosis</i> (<i>C. parapsilosis</i>)	Urinary tract infections
P36	Negative	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	Abdominal infections
P37	Negative	Negative	<i>E. coli</i>	Abdominal infections
P38	Negative	Negative	Negative	Infective endocarditis
P39	<i>C. parapsilosis</i>	Negative	<i>C. parapsilosis</i>	Other/unknown infections
P40	Negative	<i>E. coli</i>	<i>E. coli</i>	Urinary tract infections
P41	Negative	Negative	Negative	Catheter/device related infections
P42	Negative	<i>Pneumocystis jirovecii</i> (<i>P. jirovecii</i>), <i>Cytomegalo virus</i>	<i>P. jirovecii</i> , <i>Cytomegalo virus</i>	Respiratory infections
P43	Negative	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	Respiratory infections
P44	Negative	<i>Orientia tsutsugamushi</i> (<i>O. tsutsugamushi</i>)	<i>O. tsutsugamushi</i>	Skin and soft tissue infections
P45	Negative	<i>Bacteroides fragilis</i> (<i>B. fragilis</i>)	<i>B. fragilis</i>	Abdominal infections
P46	Negative	<i>Streptococcus agalactiae</i> (<i>S. agalactiae</i>)	<i>S. agalactiae</i>	Infective endocarditis
P47	Negative	Negative	Negative	Abdominal infections
P48	Negative	<i>E. coli</i>	<i>E. coli</i>	Abdominal infections
P49	Negative	<i>Prevotella oris</i> (<i>P. oris</i>)	<i>P. oris</i>	Respiratory infections
P50	Negative	Negative	<i>Mycobacterium tuberculosis</i>	Respiratory infections
P51	<i>Enterococcus faecalis</i>	<i>E. faecalis</i>	<i>E. faecalis</i>	Catheter/device related infections
P52	Negative	<i>O. tsutsugamushi</i>	<i>O. tsutsugamushi</i>	Skin and soft tissue infections
P53	<i>Salmonella enterica</i>	<i>S. enterica</i>	<i>S. enterica</i>	Infectious aneurysm
P54	Negative	<i>Bacteroides dorei</i> (<i>B. dorei</i>)	<i>B. dorei</i>	Abdominal infections
P55	Negative	<i>Mycoplasma pneumoniae</i> (<i>M. pneumoniae</i>)	<i>M. pneumoniae</i>	Respiratory infections
P56	Negative	<i>E. faecalis</i> , <i>E. faecium</i> , <i>K. pneumoniae</i>	<i>E. faecalis</i> , <i>E. faecium</i> , <i>K. pneumoniae</i>	Abdominal infections

Table S2 (Continued)

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Patient number	Blood culture	Blood mNGS	Blood tNGS	Underlying infectious syndromes
P57	<i>Candida metapsilosis</i> (<i>C. metapsilosis</i>)	Negative	<i>C. metapsilosis</i>	Infective endocarditis
P58	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	Catheter/device related infections
P59	Negative	<i>Taenia solium</i> (<i>T. solium</i>)	<i>T. solium</i>	Central nervous system infections
P60	Negative	<i>Vibrio cholerae</i> (<i>V. cholerae</i>)	<i>V. cholerae</i>	Skin and soft tissue infections
P61	<i>Brucella</i>	Negative	<i>Brucella</i>	Osteoarticular infection
P62	Negative	Negative	<i>Coxiella burnetii</i>	Catheter/device related infections
P63	Negative	Negative	<i>Aeromonas caviae</i>	Skin and soft tissue infections
P64	<i>S. sanguinis</i>	<i>S. sanguinis</i>	<i>S. sanguinis</i>	Infective endocarditis
P65	Negative	<i>S. aureus</i>	<i>S. aureus</i>	Catheter/device related infections
P66	Negative	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	Abdominal infections
P67	Negative	Negative	Negative	Infectious aneurysm
P68	Negative	Negative	<i>Bacteroides thetaiotaomicron</i> (<i>B. thetaiotaomicron</i>), <i>Parabacteroides distasonis</i>	Catheter/device related infections
P69	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	Abdominal infections
P70	Negative	<i>Legionella pneumophila</i> (<i>L. pneumophila</i>)	<i>L. pneumophila</i>	Respiratory infections
P71	Negative	<i>Prevotella bivia</i> (<i>P. bivia</i>)	<i>P. bivia</i>	Abdominal infections
P72	Negative	<i>E. faecalis</i>	<i>E. faecalis</i> , <i>Candida glabrata</i> (<i>C. glabrata</i>)	Abdominal infections
P73	Negative	<i>B. thetaiotaomicron</i>	<i>B. thetaiotaomicron</i> , <i>E. faecium</i>	Abdominal infections
P74	Negative	<i>E. coli</i>	<i>E. coli</i>	Urinary tract infections
P75	Negative	<i>Aspergillus fumigatus</i> (<i>A. fumigatus</i>)	<i>A. fumigatus</i>	Septic thrombophlebitis
P76	Negative	<i>K. pneumoniae</i> , <i>E. faecalis</i> , <i>E. faecium</i> , <i>C. glabrata</i>	<i>K. pneumoniae</i> , <i>E. faecalis</i> , <i>E. faecium</i> , <i>C. glabrata</i>	Abdominal infections
P77	<i>S. enterica</i>	<i>S. enterica</i>	<i>S. enterica</i>	Infective endocarditis
P78	Negative	<i>E. faecium</i>	<i>E. faecium</i>	Abdominal infections
P79	Negative	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	Abdominal infections
P80	Negative	<i>Proteus mirabilis</i>	<i>P. mirabilis</i>	Urinary tract infections

^a, including native valve endocarditis, prosthetic valve endocarditis and pacemaker-related endocarditis; ^b, including infections related to central line catheters, artificial blood vessels and stent implantation. mNGS, metagenomic next-generation sequencing; tNGS, targeted next-generation sequencing; NA, not applicable; BSI, bloodstream infection.

Table S3 Consistency between blood tNGS results and microbes detected by culture or mNGS in other specimens

Patient number	Underlying infectious syndromes	Blood tNGS results	Other specimens		
			Specimen type	Culture results	mNGS results
P16	Abdominal infections	<i>Klebsiella pneumoniae</i> (<i>K. pneumoniae</i>)	Pus	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>
P18	Abdominal infections	<i>K. pneumoniae</i>	Pus	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>
P21	Abdominal infections	<i>K. pneumoniae</i>	Pus	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>
P23	Skin and soft tissue infections	<i>Staphylococcus aureus</i> (<i>S. aureus</i>)	Pus	<i>S. aureus</i>	<i>S. aureus</i>
P24	Skin and soft tissue infections	<i>Aeromonas veronii</i> (<i>A. veronii</i>)	Pus	Negative	<i>A. veronii</i>
P26	Skin and soft tissue infections	<i>Vibrio vulnificus</i> (<i>V. vulnificus</i>)	Pus	Negative	<i>V. vulnificus</i>
P27	Urinary tract infections	<i>Escherichia coli</i> (<i>E. coli</i>)	Urine	Negative	<i>E. coli</i>
P43	Respiratory infections	<i>K. pneumoniae</i>	Sputum	Negative	<i>K. pneumoniae</i>
P48	Abdominal infections	<i>E. coli</i>	Pus	Negative	<i>E. coli</i>
P49	Respiratory infections	<i>Prevotella oris</i> (<i>P. oris</i>)	Pleural fluid	Negative	<i>P. oris</i>
P50	Respiratory infections	<i>Mycobacterium tuberculosis</i> (<i>M. tuberculosis</i>)	Pleural fluid	<i>M. tuberculosis</i>	<i>M. tuberculosis</i>
P52	Skin and soft tissue infections	<i>Orientia tsutsugamushi</i> (<i>O. tsutsugamushi</i>)	Pus	Negative	<i>O. tsutsugamushi</i>
P55	Respiratory infections	<i>Mycoplasma pneumoniae</i> (<i>M. pneumoniae</i>)	Sputum	Negative	<i>M. pneumoniae</i>
P56	Abdominal infections	<i>Enterococcus faecalis</i> (<i>E. faecalis</i>), <i>Enterococcus faecium</i> (<i>E. faecium</i>), <i>K. pneumoniae</i>	Bile	<i>E. faecalis</i>	<i>E. faecalis</i> , <i>K. pneumoniae</i>
P59	Central nervous system infections	<i>Taenia solium</i> (<i>T. solium</i>)	Cerebrospinal fluid	Negative	<i>T. solium</i>
P60	Skin and soft tissue infections	<i>Vibrio cholerae</i> (<i>V. cholerae</i>)	Pus	Negative	<i>V. cholerae</i>
P62	Catheter/device related infections ^a	<i>Coxiella burnetii</i> (<i>C. burnetii</i>)	Pus	Negative	<i>C. burnetii</i>
P63	Skin and soft tissue infections	<i>Aeromonas caviae</i> (<i>A. caviae</i>)	Pus	<i>A. caviae</i>	<i>A. caviae</i>
P70	Respiratory infections	<i>Legionella pneumophila</i> (<i>L. pneumophila</i>)	Sputum	Negative	<i>L. pneumophila</i>
P73	Abdominal infections	<i>Bacteroides thetaiotaomicron</i> (<i>B. thetaiotaomicron</i>), <i>E. faecium</i>	Peritoneal fluid	<i>E. faecium</i> , <i>E. coli</i>	<i>B. thetaiotaomicron</i> , <i>E. faecium</i> , <i>K. pneumoniae</i> , <i>Candida albicans</i>
P78	Abdominal infections	<i>E. faecium</i>	Peritoneal fluid	<i>E. faecium</i>	NA
P79	Abdominal infections	<i>K. pneumoniae</i>	Pus	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>
P80	Urinary tract infections	<i>Proteus mirabilis</i> (<i>P. mirabilis</i>)	Urine	Negative	<i>P. mirabilis</i>

^a, including infections related to central line catheters, artificial blood vessels and stent implantation. tNGS, targeted next-generation sequencing; mNGS, metagenomic next-generation sequencing; NA, not applicable.