Supplementary Table 1. Results according to species of direct identification using an in-house MALDI-TOF MS protocol performed on 505 monomicrobial positive pediatric blood culture bottles

| Colony identification | n     | Correct ID Score >2 n (%) | Correct ID Score 1.7-1.99 n (%) | Score <1.7 n (%) | False ID n (%) |
|-----------------------|-------|---------------------------|---------------------------------|------------------|----------------|
| Enterobacterales      | 113   | 104 (92)                  | 9 (8)                           | 0                | 0              |
| *Klebsiella pneumoniae* | 49    | 46 (94)                   | 3 (6)                           | 0                | 0              |
| *Eschericia coli*     | 30    | 30 (100)                  | 0                               | 0                | 0              |
| *Salmonella spp.*     | 10    | 9 (90)                    | 1 (150)                         | 0                | 0              |
| *Enterobacter cloacae* | 9     | 7 (78)                    | 2 (22)                          | 0                | 0              |
| *Serratia marcescens* | 5     | 4 (80)                    | 1 (20)                          | 0                | 0              |
| *Klebsiella oxytoca*  | 4     | 3 (75)                    | 1 (25)                          | 0                | 0              |
| *Enterobacter aerogenes* | 2    | 2 (100)                   | 0                               | 0                | 0              |
| *Proteus mirabilis*   | 1     | 1 (100)                   | 0                               | 0                | 0              |
| *Pantoea eucrina*     | 1     | 0                         | 1 (100)                         | 0                | 0              |
| *Raoultella ornithinolytica* | 1  | 1 (100)                   | 0                               | 0                | 0              |
| *Klebsiella aerogenes* | 1    | 1 (100)                   | 0                               | 0                | 0              |
| Colony identification                  | n  | Correct ID Score >2 n (%) | Correct ID Score 1.7-1.99 n (%) | Score <1.7 n (%) | False ID n (%) |
|----------------------------------------|----|---------------------------|----------------------------------|------------------|----------------|
| Non-fermenting gram-negative rods      |    |                           |                                  |                  |                |
| *Pseudomonas aeruginosa*               | 19 | 18 (95)                   | 1 (5)                            | 0                | 0              |
| *Ochrobacterium intermedium*           | 2  | 2 (100)                   | 0                                | 0                | 0              |
| *Acinetobacter baumannii*              | 1  | 1 (100)                   | 0                                | 0                | 0              |
| *Achromobacter xylosoxidans*           | 1  | 1 (100)                   | 0                                | 0                | 0              |
| *Chryseobacterium indologenes*         | 1  | 1 (100)                   | 0                                | 0                | 0              |
| *Elizabethkingia meningoseptica*       | 1  | 0                         | 1 (100)                          | 0                | 0              |
| *Sphingomonas paucimobilis*            | 1  | 0                         | 0                                | 1 (100)          | 0              |
| *Stenotrophomonas maltophilia*         | 1  | 2 (100)                   | 0                                | 0                | 0              |
| Colony identification                        | n | Correct ID Score >2 n (%) | Correct ID Score 1.7-1.99 n (%) | Score <1.7 n (%) | False ID n (%) |
|---------------------------------------------|---|---------------------------|---------------------------------|------------------|----------------|
| Fastidious gram-negative coccobacilli       | 8 | 2 (25)                    | 3 (38)                          | 3 (38)           | 0              |
| *Haemophilus influenza*                     | 3 | 0                         | 2 (67)                          | 1 (33)           | 0              |
| *Moraxella catarrhalis*                    | 2 | 2 (100)                   | 0                               | 0                | 0              |
| *Moraxella osloensis*                      | 2 | 0                         | 1 (50)                          | 1 (50)           | 0              |
| *Campylobacter coli*                       | 1 | 0                         | 0                               | 1 (100)          | 0              |
| Colony identification | n  | Correct ID Score >2 n (%) | Correct ID Score 1.7-1.99 n (%) | Score <1.7 n (%) | False ID n (%) |
|-----------------------|----|---------------------------|-------------------------------|-----------------|---------------|
| Staphylococci         | 256| 130 (51)                  | 116 (45)                      | 10 (4)          | 0             |
| _Staphylococcus epidermidis_ | 124| 30 (24)                   | 84 (68)                       | 10 (8)          | 0             |
| _Staphylococcus hominis_ | 52 | 42 (81)                   | 10 (19)                       | 0               | 0             |
| _Staphylococcus aureus_ | 41 | 34 (83)                   | 7 (17)                        | 0               | 0             |
| _Staphylococcus capitis_ | 21 | 13 (62)                   | 8 (38)                        | 0               | 0             |
| _Staphylococcus haemolyticus_ | 12 | 8 (67)                    | 4 (33)                        | 0               | 0             |
| _Staphylococcus warneri_ | 2  | 1 (50)                    | 1 (50)                        | 0               | 0             |
| _Staphylococcus saprophyticus_ | 1  | 0                         | 1 (100)                       | 0               | 0             |
| _Staphylococcus caprae_ | 1  | 0                         | 1 (100)                       | 0               | 0             |
| _Staphylococcus pettenkofei_ | 1  | 1 (100)                   | 0                             | 0               | 0             |
| _Staphylococcus spp._  | 1  | 1 (100)                   | 0                             | 0               | 0             |
| Colony identification                        | n  | Correct ID Score >2 n (%) | Correct ID Score 1.7-1.99 n (%) | Score <1.7 n (%) | False ID n (%) |
|---------------------------------------------|----|---------------------------|----------------------------------|------------------|----------------|
| Streptococci                                | 61 | 44 (72)                   | 15 (25)                          | 2 (3)            | 5 (8)          |
| Viridans group streptococci                 | 24 | 16 (67)                   | 8 (33)                           | 1 (9)            | 5 (21)         |
| *Enterococcus faecalis*                     | 13 | 12 (92)                   | 1 (8)                            | 0                | 0              |
| *Streptococcus pneumoniae*                  | 11 | 6 (55)                    | 4 (36)                           | 1 (9)            | 0              |
| Group A *Streptococcus*                     | 4  | 3 (75)                    | 1 (25)                           | 0                | 0              |
| Group B *Streptococcus*                     | 4  | 3 (75)                    | 1 (25)                           | 0                | 0              |
| *Abiotrophia defectiva*                     | 2  | 1 (50)                    | 0                                | 1 (50)           | 0              |
| *Enterococcus faecium*                      | 1  | 1 (100)                   | 0                                | 0                | 0              |
| *Streptococcus gallolyticus*                | 1  | 1 (100)                   | 0                                | 0                | 0              |
| *Granulicatella adiacens*                   | 1  | 1 (100)                   | 0                                | 0                | 0              |
| Colony identification                | n | Correct ID Score >2 n (%) | Correct ID Score 1.7-1.99 n (%) | Score <1.7 n (%) | False ID n (%) |
|-------------------------------------|---|---------------------------|--------------------------------|------------------|---------------|
| Gram-positive rods                  | 19| 6 (32)                    | 6 (32)                         | 7 (37)           | 0             |
| Bacillus spp.                       | 8 | 5 (63)                    | 3 (38)                         | 0                | 0             |
| Corynebacterium spp.                | 6 | 1 (17)                    | 1 (17)                         | 4 (67)           | 0             |
| Actinomyces spp                     | 2 | 0                         | 0                              | 2 (100)          | 0             |
| Rothia dentocariosa                 | 1 | 0                         | 0                              | 1 (100)          | 0             |
| Leuconostoc mesenteroides           | 1 | 0                         | 1 (100)                        | 0                | 0             |
| Microbacterium lacticum             | 1 | 0                         | 1 (100)                        | 0                | 0             |
| Colony identification           | n  | Correct ID Score >2 n (%) | Correct ID Score 1.7-1.99 n (%) | Score <1.7 n (%) | False ID n (%) |
|--------------------------------|----|---------------------------|----------------------------------|------------------|----------------|
| Other gram-positive bacteria   | 13 | 11 (85)                   | 1 (8)                            | 1 (8)            | 0              |
| *Micrococcus luteus*           | 12 | 10 (83)                   | 1 (8)                            | 1 (8)            | 0              |
| *Kokuria kristinae*            | 1  | 1 (100)                   | 0                                | 0                | 0              |
| Colony identification | n  | Correct ID Score >2 n (%) | Correct ID Score 1.7-1.99 n (%) | Score <1.7 n (%) | False ID n (%) |
|-----------------------|----|---------------------------|---------------------------------|-----------------|----------------|
| Candida spp.          | 8  | 0                         | 5 (63)                          | 3 (38)          | 0              |
| Candida albicans      | 5  | 0                         | 3 (60)                          | 2 (40)          | 0              |
| Candida metapsilosis  | 1  | 0                         | 1 (100)                         | 0               | 0              |
| Candida parapsilosis  | 1  | 0                         | 0                               | 1 (100)         | 0              |
| Candida tropicalis    | 1  | 0                         |                                 | 1 (100)         | 0              |
Supplementary Table 2. Unsuccessful direct identification using an in-house MALDI-TOF MS protocol

| Colony ID | DIMT | DIMT score | Clinical significance |
|-----------|------|------------|-----------------------|
| Abiotrophia defectiva | NOIDP | NPD | Contamination |
| Candida tropicalis  
Staphylococcus hominis | Candida tropicalis | 1.38 | Significant |
| Corynebacterium afermentans | Corynebacterium afermentans | 1.47 | Contamination |
| Staphylococcus epidermidis | Staphylococcus epidermidis | 1.54 | Contamination |
| Staphylococcus epidermidis | Staphylococcus epidermidis | 1.57 | Contamination |
| Sphingomonas paucimobilis  
Sphingobium chlorophenolicum | | 1.35 | Contamination |
| Campylobacter coli | NOIDP | NPD | Significant |
| Corynebacterium spp. | NOIDP | NPD | Contamination |
| Staphylococcus epidermidis | Staphylococcus epidermidis | 1.59 | Significant |
| Brevundimonas diminuta Acidovorax temperans | Gordonia rubripertincta | 1.26 | Contamination |
|---------------------------------------------|------------------------|------|---------------|
| *Staphylococcus epidermidis* | *Staphylococcus epidermidis* | 1.53 | Significant |
| *Candida parasilopsis* | *Candida parasilopsis* | 1.48 | Significant |
| *Haemophilus influenzae* | NOIDP | NPD | Significant |
| *Candida albicans* | *Candida albicans* | 1.53 | Significant |
| *Actinomyces odontolyticus* | NOIDP | NPD | Contamination |
| *Streptococcus pneumoniae* | NOIDP | NPD | Significant |
| *Rothia dentocariosa* | NOIDP | NPD | Contamination |
| *Moraxella osloensis* | *Clostridium cochlearium* | 1.34 | Contamination |
| *Corynebacterium spp* | *Corynebacterium afermentans* | 1.46 | Contamination |
**Supplementary Table 3.** Optimal de-escalating and escalating interventions identified in clinically significant bacteremic episodes based on DIMT

| De-escalation | Glycopeptide or 3G/4G-cephalosporins to ampicillin for *Enterococcus faecalis*
| Piperacillin-tazobactam to ceftriaxone for *Haemophilus influenzae*
| Glycopeptide or 3G-cephalosporins to penicillin for beta-hemolytic streptococci
| Amphotericin B or echinocandin to fluconazole for *Candida albicans*

| Escalation | 3G-cephalosporin to glycopeptides for *Enterococcus faecium*
| Cloxacillin to glycopeptides for coagulase-negative staphylococci
| Ampicillin + gentamicin to piperacillin-tazobactam for *Pseudomonas aeruginosa*
| 3G-cephalosporins or piperacillin-tazobactam to meropenem for AmpC beta-lactamase producers
| Ampicillin + gentamicin to meropenem for *Klebsiella pneumoniae* |
**Supplementary Table 4.** Treatment optimization in blood stream infections prompted by direct identification by MALDI-TOF

| Age   | Underlying condition | Sepsis source | Empirical therapy | DIMT result | Changed prompted by DIMT |
|-------|----------------------|---------------|-------------------|-------------|-------------------------|
| 5 years | Vesicoureteral reflux and recurrent urinary tract infection | Urosepsis | Ceftriaxone | *E. faecalis* | Narrowed to ampicillin |
| 5 months | Coffin-Siris syndrome, intubated & ventilated, long PICU stay | VAP | Vancomycin | *P. aeruginosa* | Meropenem added |
| 12 months | Tufting enteropathy, PICC in place, TPN-dependent | CLABSI | Vancomycin | *E. cloacae* | Meropenem added |
| 7 weeks | Lung malformation, enteroviral myocarditis, intubated & ventilated, long NICU stay | VAP | Vancomycin + pip-taz | *S. marcescens* | Escalated to meropenem |
| 13 years | Stuve-Wiedemann syndrome, bone dysplasia, severe scoliosis | HAP | Pip-taz | non-typeable *H. influenzae* | Narrowed to ceftriaxone |
| Duration  | Diagnosis/Procedure                                                                 | Infection Type     | Antibiotics          | Outcome                        |
|-----------|-------------------------------------------------------------------------------------|--------------------|----------------------|--------------------------------|
| 3 weeks   | Anorectal malformation, colostomy                                                   | Surgical wound    | Meropenem            | $E.\ faecalis$                  |
|           |                                                                                     | infection          |                      | Ampicillin added                |
| 16 years  | Duchenne muscular PICC & tracheostomy in place, non-invasive ventilation in PICU     | SWAS               | Pip-taz              | $E.\ cloacae$                   |
|           |                                                                                     |                    |                      | Escalated to meropenem           |
| 8 years   | Short bowel syndrome, port-a-cath in place, TPN-dependent                            | CLABSI             | Vancomycin + ceftriaxone | $E.\ cloacae$                   |
|           |                                                                                     |                    |                      | Escalated to meropenem           |
| 7 weeks   | Prematurity, NEC, laparotomy, Ileostomy in place, long NICU stay                     | VLOGBSS            | Vancomycin + pip-taz | $S.\ agalactiae$                |
|           |                                                                                     |                    |                      | Narrowed to penicillin + gentamicin |
| 5 months  | Short bowel syndrome, PICC & ileostomy in place, TPN-dependent                      | CLABSI             | Vancomycin            | $E.\ aerogenes$                 |
|           |                                                                                     |                    |                      | Meropenem added                  |
| 6 weeks   | Pyloric atresia, laparotomy, PICC in place, long NICU stay                           | CLABSI             | Cloxacillin + gentamicin | $S.\ capitis$                   |
|           |                                                                                     |                    |                      | Escalated to vancomycin          |
| 4 weeks   | CHD, cardiac catheterization, CVC in place, intubated & ventilated, long PICU stay  | SWAS               | Teicoplanin + gentamicin | $E.\ faecalis$                  |
|           |                                                                                     |                    |                      | Narrowed to ampicillin + gentamicin |
| Duration | Diagnosis Details | Infection Details | Antimicrobial Therapy | Antibiotic Adjustment |
|----------|-------------------|------------------|-----------------------|----------------------|
| 6 years  | Previously healthy | Acute Osteomyelitis | Ceftriaxone | \(S.\text{ pyogenes}\) Narrowed to penicillin + clindamycin |
| 11 years | Acute lymphoblastic leukemia, port-a-cath in place, neutropenia, ongoing MSSA sepsis | CLABSI | Cefazolin + amikacin | \(E.\text{ cloaca}\) Meropenem added |
| 3 weeks  | Hirschsprung's disease Intestinal resection, Ileostomy, PICC in place, TPN | SWAS | Vancomycin + meropenem | \(E.\text{ faecalis}\) Narrowed to ampicillin + gentamicin |
| 5 months | Omenn's syndrome, recurrent enterococcal sepsis, sepsis PICC in place, TPN | CLABSI | Ampicillin + gentamicin | \(P.\text{ aeruginosa}\) Escalated to pip-taz |
| 3 months | Hirschsprung's disease Intestinal resection, Ileostomy, PICC in place, TPN | SWAS | Vancomycin + gentamicin | \(E.\text{ faecalis}\) Narrowed to ampicillin + gentamicin |
| 5 months | Multiple neonatal co-morbidities, bronchiolitis, epilepsy, PICC in place, long PICU stay | SWAS | Off antibiotics | \(P.\text{ aeruginosa}\) Started on pip-taz |
| Age      | Diagnosis                                             | Treatment                          | Pathogen          | Outcome                      |
|----------|-------------------------------------------------------|------------------------------------|-------------------|------------------------------|
| 16 months | Congenital muscular dystrophy, viral exacerbation CLD PEG feeding tube in place | SWAS | Ceftriaxone | E. faecalis | Narrowed to ampicillin |
| 1 month  | Ileal atresia, necrotic bowel, laparotomy, adhesiolysis | Peritonitis | Vancomycin + gentamicin + metronidazole | E. faecalis | Narrowed to ampicillin + gentamicin + metronidazole |
| 2 months | Ileal atresia, laparotomy, PICC in place, TPN       | CLABSI | Ampicillin + gentamicin | K. pneumoniae | Escalated to meropenem |
| 11 months | Acute myeloid leukemia, induction chemotherapy, port-a-cath, non-neutropenic fever | CLABSI | Ceftriaxone | E. faecium | Escalated to teicoplanin |
| 2 years  | Short bowel syndrome, PICC in place, TPN            | CLABSI | Off antibiotics | E. faecalis | Started on ampicillin + gentamicin |
| 16 months | Previously healthy                                   | Infected burn wound | Off antibiotics | S. aureus | Started on cloxacillin |
| 23 months | Brain tumor, port-a-cath, non-neutropenic sepsis    | CLABSI | Vancomycin + Ceftriaxone | S. marcescens | Escalated to meropenem |
| Age       | Preceding Condition       | Clinical Status | Antibiotics  | Pathogen      | Initial Treatment |
|-----------|---------------------------|-----------------|--------------|---------------|-------------------|
| 3 months  | IUGR                      | VLOGBSS         | Off antibiotics | *S. agalactiae* | Started on ampicillin |
| 10 months | Previously healthy         | OB              | Off antibiotics | *S. pneumoniae* | Started on ceftriaxone |

DIMT: Direct identification by MALDI-TOF. ICU: Pediatric Intensive Care Unit; VAP: Ventilator-associated pneumonia; PICC: Peripherally inserted central catheter; TPN: Total parenteral nutrition; CLABSI: Central line associated blood stream infection; NICU: Neonatal Intensive Care Unit; HAP: Hospital-acquired pneumonia; Pip-taz: Piperacillin-tazobactam; SWAS: Sepsis without an apparent source; NEC: Necrotizing enterocolitis; VLOGBSS: Very late-onset group B *Streptococcus* sepsis; CHD: Congenital heart disease; CVC: Central venous catheter; MSSA: Methicillin-susceptible *Staphylococcus aureus*; CLD: Chronic lung disease; PEG: percutaneous endoscopic gastrostomy; IUGR: Intrauterine growth restriction.
**Supplementary Table 5.** Missed opportunities to optimize treatment in blood stream infections based on direct identification by MALDI-TOF

| Age   | Underlying condition                                                                 | Sepsis source | Empirical therapy          | DIMT result | Treatment after notification of DIMT |
|-------|--------------------------------------------------------------------------------------|---------------|-----------------------------|-------------|-------------------------------------|
| 12 months | Autoimmune enteropathy, PICC in place, TPN-dependent                                 | CLABSI        | Cefepime                    | *E. faecalis* | Switched to teicoplanin             |
| 12 days | 31 weeker, gastric perforation, laparotomy                                            | Peritonitis   | Teicoplanin + meropenem     | *C. albicans* | Amphotericin B added               |
| 5 years | Ohtahara syndrome, recurrent LRTI, recent meropenem course, PICC in place, ARF, hemodialysis | CLABSI        | Vancomycin + cefepime      | *E. faecalis* | Unchanged                           |
| 5 weeks | Cystic fibrosis, meconium ileus & pseudocyst, laparotomy, ileostomy                   | Peritonitis   | Vancomycin + pip-taz        | *C. albicans* | Amphotericin B added               |
| 3 years | ACS, hydrocephalus, spina bifida, neurogenic bladder, ongoing ESBL bacteremia         | Urosepsis     | Ertapenem                   | *C. albicans* | Caspofungin added                   |
| Duration  | Condition(s)                                                                 | Site(s)                       | Treatment(s) | Organism(s)                             | Outcome(s)                  |
|-----------|------------------------------------------------------------------------------|-------------------------------|--------------|-----------------------------------------|-----------------------------|
| 17 months | Boring Opitz syndrome, Upper GI surgery, broad-spectrum antibiotic treatment, PEG feeding tube in place | Abdominal collection          | Meropenem    | *C. albicans*                           | Amphotericin B added        |
| 4 weeks   | 28 weeker, CLD, NEC, laparotomy, ileostomy                                   | Abdominal collection          | Pip-taz      | *S. marcescens*                         | Unchanged                   |
| 4 weeks   | 31 weeker, anorectal malformation, abdominal surgery, colostomy              | LONS                          | Cloxacillin + Gentamicin            | *S. marcescens*                          | Unchanged                   |
| 3 months  | Congenital ichthyosis, primary immunodeficiency                              | SWAS                          | Vancomycin + meropenem              | *E. faecalis*                           | Unchanged                   |
| 2 years   | Previously healthy                                                           | Lymphadenitis                 | Clindamycin  | *S. pyogenes*                           | Unchanged                   |
| 4 months  | SCID, pressure ulcer, ongoing *P. aeruginosa* bacteremia                    | SWAS                          | Ceftazidime-avibactam               | *E. faecalis*                           | Vancomycin added            |
| 4 months  | Short bowel syndrome, PICC in place, TPN-dependent, long NICU stay           | SWAS                          | Cloxacillin + gentamicin            | *E. faecalis*                           | Switched to vancomycin + gentamicin |
| 4 months | Prematurity, cholestatic jaundice, laparotomy, stoma closure, PICC in place, long NICU stay | SWAS | Vancomycin + pip-taz | *E. cloacae* | Unchanged |

DIMT: Direct identification by MALDI-TOF. PICC: Peripherally inserted central catheter; TPN: Total parenteral nutrition; CLABSI: Central line associated blood stream infection; LRTI: Lower respiratory tract infection; ARF: Acute renal failure; Pip-taz: Piperacillin-tazobactam; ESBL: Extended spectrum beta-lactamase; PEG: percutaneous endoscopic gastrostomy; CLD: Chronic lung disease; NEC: Necrotizing enterocolitis; LONS: Late-onset neonatal sepsis; SWAS: Sepsis without an apparent source; SCID: Severe combined immunodeficiency; PICC: Peripherally inserted central catheter; TPN: Total parenteral nutrition; NICU: Neonatal Intensive Care Unit.