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Clinical and metagenomic predicted antimicrobial resistance in pediatric critically ill patients with infectious diseases in a single center of Zhejiang

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Abstract

Background Antimicrobial resistance (AMR) poses a significant threat to pediatric health; therefore, precise identification of pathogens as well as AMR is imperative. This study aimed at comprehending antibiotic resistance patterns among critically ill children with infectious diseases admitted to pediatric intensive care unit (PICU) and to clarify the impact of drug-resistant bacteria on the prognosis of children.

Methods This study retrospectively collected clinical data, identified pathogens and AMR from 113 children's who performed metagenomic next-generation sequencing for pathogen and antibiotic resistance genes identification, and compared the clinical characteristic difference and prognostic effects between children with and without AMR detected.

Results Based on the presence or absence of AMR test results, the 113 patients were divided into Antimicrobial resistance test positive group (AMRT+, $n=44$) and Antimicrobial resistance test negative group (AMRT-, $n=69$). Immunocompromised patients (50% vs. 28.99%, $P=0.0242$) and patients with underlying diseases (70.45% vs. 40.58%, $P=0.0019$) were more likely to develop resistance to antibiotics. Children in the AMRT + group showed significantly increased C-reaction protein, score of pediatric sequential organ failure assessment and pediatric risk of mortality of children and longer hospital stay and ICU stay in the AMRT + group compared to the AMRT+- group ($P<0.05$). Detection rate of Gram-negative bacteria was significantly higher in the AMRT + group rather than Gram-positive bacteria ($n=45$ vs. 31), in contrast to the AMRT- group ($n=10$ vs. 36). Cephalosporins, β-lactams/β-Lactamase inhibitors, carbapenems and sulfonamides emerged as the most common types of drug resistance in children. Resistance rates to these antibiotics exhibited considerable variation across common pathogens, including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

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Conclusions The development of drug resistance in bacteria will significantly affect the prognosis of patients. The significant differences in drug resistance of common pathogenic bacteria indicate that identification of drug resistance is important for the rational use of antibiotics and patient prognosis.

Keywords Antimicrobial resistance, Pediatric, Metagenomic next-generation sequencing, Antibiotic susceptibility testing, Infectious disease

Background

The burden of multidrug-resistant bacteria (MDRB) on health systems is a global problem that has a significant impact on human health and socioeconomics [1]. The increase and spread of MDRB has made standardized treatments ineffective [2]. Patients with MDRB infection often have complex conditions and are difficult to cure. They need to be treated with more advanced antimicrobial drugs, which imposes a heavy economic burden on patients. MDRB can be transmitted through contact with contaminated hands and objects, which can easily cause hospital infections, prolong patients' hospital stays, increase medical costs, and even lead to death [3, 4]. Children have lower immunity and are more susceptible to get infections. Clear diagnosis of pathogens is of great significance for the prevention, diagnosis and treatment of diseases. Due to differences in the types of diseases and the use of antibiotics in different regions, the epidemiological characteristics and resistance characteristics of MDRB are different.

The most commonly used method for clinical MDRB detection is culture-dependent antibiotic susceptibility testing (AST) [5]. The processes from sample collection to obtain drug susceptibility phenotype involves three steps: bacterial and fungal growth, taxonomic identification of isolated bacterial and fungal colonies, and AST. After the sample is collected, it will be sent to the laboratory for bacterial or fungal culture. Detection of bacterial growth in culture bottles can take up to 5 days, but often occurs within the first 24 h of incubation; while fungal culture take up at least 2–3 days. Next, pathogen identification typically takes another 24 h. Finally, AST on pure bacterial and fungal colonies typically also requires 4–24 h [6]. The advantages of AST are convenience, sensitivity, repeatability and low cost. However, this type of method requires long-term cultivation and is limited to the detection of bacterial resistance phenotype, and cannot detect resistance genes [7]. In recent years, metagenomic next-generation sequencing (mNGS) technology has been developed. It can directly obtain nucleic acid information of pathogenic microorganisms from clinical samples, and can simultaneously detect resistance genes and gene mutations [8]. However, there is currently insufficient clinical understanding of the accuracy of mNGS testing in predicting antimicrobial resistance (AMR), making it difficult to provide theoretical support for clinical antibiotic decision-making.

This study retrospectively analyzed the antimicrobial resistance detected by mNGS and AST in children with infectious diseases in the pediatric intensive care unit (PICU), and explored their effects on prognosis.

Methods

Study design

We retrospectively included children with severe infection in Pediatric Intensive Care Unit (PICU) of Children's Hospital of Zhejiang University School of Medicine from January 2022 to March 2024. The inclusion criteria are: (1) patients suspected with infectious disease; (2) patients who performed mNGS for pathogen and antibiotic resistance genes (ARG) identification. Exclusion criteria include: (1) without complete clinical records; (2) No conventional microbiological test (CMT) results. For cases where multiple samples were tested by mNGS, only the first tested sample was included.

Conventional microbiological tests

The performed conventional microbiological test (CMT) methods including bacteria and fungi culture, smearing, (1,3)-β-D-glucan (G) test, T-spot, PCR of respiratory virus (including influenza A virus, influenza B virus, parainfluenza virus, adenovirus, respiratory syncytial virus, human metapneumovirus, rhinovirus, coronavirus, and bocavirus), and antibody or nucleic acid amplification tests for *Mycoplasma pneumoniae*.

Antibiotic susceptibility testing for bacteria and fungi

Blood and cerebrospinal fluid (CSF) samples were cultivated using children's aerobic microbe culture bottles, and positive smear results were transferred to Columbia blood agar medium, chocolate medium, or Shabao-weak medium for overnight incubation at 35 °C. Bronchoalveolar lavage fluid (BALF) samples were directly inoculated into the aforementioned medium and incubated overnight at 35 °C. The cultivated strains were identified through matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Bruker, USA). An automatic microbial identification and antibiotic sensitivity analysis system VITEK$^{@}$2 compact (bioMérieux, France) was employed in the antibiotic susceptibility testing (AST), and the results were interpreted in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [9].

When testing the drug resistance of bacteria, the detection of antibiotics to which they are naturally resistant is excluded. The drug resistance rate of bacteria is calculated based on the drug sensitivity results. The formula for calculating the drug resistance rate to a certain antibiotic or a certain class of antibiotics is: number of positive result sample/(number of positive result sample + number of negative result sample).

Metagenomic next-generation sequencing

Based on the patient's infection site and symptoms, the clinician selects the appropriate sample type for mNGS testing. These include BALF, peripheral blood, CSF, sputum, pleural fluid (PE), and throat swabs. Blood sample were centrifuged at 1900 g and 4℃ for 10 min, and samples of other body fluids were centrifuged at 12,075 g and 4℃ for 5 min. Sputum samples need to be liquefied. (1) DNA extraction: genomic DNA was extracted after host depletion using the PathoXtract® WYXM03202S universal pathogen enrichment extraction kit (WillingMed, Beijing, China). (2) Library construction and sequencing: DNA libraries were constructed with the Illumina® DNA Prep (M) Tagmentation kit (20018705; Illumina, San Diego, USA). Library concentration was quantified using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). A 75-bp single-end sequencing was performed with a NextSeqTM 550Dx sequencer (Illumina, San Diego, USA), with at least 20 million sequencing reads obtained for each sample. (3) Data analysis: The FASTQ-format data obtained by sequencing was processed with Trimmomatic [10] software to filter out low-quality sequences, contaminated adapters, duplicated reads and reads shorter than 36 bp. Then the sequences were compared with the human reference genome GRCh37 (hg19) using Bowtie2 to remove human sequences [11]. For taxonomic classification and identification of microbial reads, we utilized Kraken2 software and non-redundant nucleotide sequences database of National Center for Biotechnology Information (NCBI), and the pathogens were classified into bacteria, fungi, virus and parasites [12]. To interpret the results of mNGS, the following criteria were applied to report the positive pathogens. Reads per ten million (RPTM) was used to quantify pathogen abundance. Bacteria and fungi with RPTM ≥ 20 [13], viruses with RPTM ≥ 3, and special pathogens (including *Cryptococcus*, *Mycobacterium*, *Mycoplasma*, *Chlamydia*, *Legionella*, and parasites) with RPTM ≥ 1, was identified as positive [14, 15].

Statistics analysis

Independent variables were expressed as counts and percentages. Continuous variables with normal distribution were expressed as mean ± standard error (SE). Statistical analyses were performed using Prism 9 (GraphPad, La Jolla, CA). The Wilcoxon-Mann-Whitney test was used for comparisons between groups, and chi-square test was used for categorical variables. *P*-values less than 0.05 were considered statistical significance.

Results

Patient characteristics

Based on the established inclusion and exclusion criteria, a total of 113 patients were retrospectively enrolled. According to the mNGS and CMT results, 44 patients (38.94%) were classified into the Antimicrobial resistance test positive (AMRT+) group, while the remaining 69 patients were belonged to the Antimicrobial resistance test negative (AMRT-) group. The demographic and baseline characteristics of the enrolled patients were detailed in Table 1. No significant differences were observed between the two groups regarding gender and age. However, the body mass index (BMI) of AMRT + patients was slightly lower than that of AMRT- patients (14.80 ± 2.58 vs. 16.12 ± 3.53, $P = 0.0411$). In comparison to AMRT- patients, a significantly higher proportion of AMRT + cases exhibited immunosuppression (50% vs. 28.99%, $P = 0.0242$) and had underlying disease (70.45% vs. 40.58%, $P = 0.0019$), with leukemia being predominant among them. Most clinical features did not differ significantly between both groups, except for a notable increase of C-reaction protein (CRP) level among AMRT + children compared to the AMRT- cases (67.69 ± 80.16 vs. 29.18 ± 46, $P = 0.0025$). Scores measuring disease severity varied significantly between groups: pediatric sequential organ failure assessment (pSOFA) and pediatric risk of mortality (PRISM) were higher in the AMRT + group, whereas pediatric critical illness score (PCIS) were elevated in the AMRT- group ($P < 0.05$). Additionally, both length of hospital stay (LOHS) and ICU duration were significantly longer for those in AMRT + group compared to those in AMRT- group. However, mortality rates did not show any significant difference between these two groups.

Among these patients, 28 experienced multi-site infections. Pulmonary infections ($n = 79$) were most prevalent, followed by bloodstream infections ($n = 29$) and central nervous system infections ($n = 25$). Furthermore, a markedly higher incidence rate of pulmonary infection (88.64% vs. 57.97%, $P = 0.0005$) and central nervous system infection (45.45% vs. 7.25%, $P < 0.0001$) was noted within the AMRT + group compared to the AMRT- group (Table 2). The most frequently observed disease types included severe pneumonia ($n = 53$), sepsis ($n = 34$), septic shock ($n = 25$), pneumonia ($n = 24$) and encephalitis ($n = 19$), with incidence of these first three conditions being substantially higher in AMRT + group compared to AMRT- group ($P < 0.05$). Conversely, the proportion of encephalitis was found to be significantly greater in the

Table 1 Characteristic of patients in AMRT + and AMRT- group

Group	AMRT+ (*N* = 44) [a]	AMRT- (*N* = 69) [a]	*P*-value
Gender-boy, *n* (%)	25 (56.82%)	41 (59.42%)	0.7844
Age, mean ± SD	80.34 ± 55.10	71.20 ± 50.07	0.4119
Immunosuppression, *n* (%)	22 (50%)	20 (28.99%)	0.0242
Underlying disease, *n* (%)	31 (70.45%)	28 (40.58%)	0.0019
Leukemia, *n* (%)	17 (38.64%)	12 (17.39%)	0.0117
Symptoms			
Fever, *n* (%)	42 (95.45%)	59 (85.51%)	0.0942
Cough, *n* (%)	17 (38.64%)	15 (21.74%)	0.0519
Clinical feature, mean ± SD			
BMI	14.80 ± 2.58	16.12 ± 3.53	0.0411
pSOFA	5.27 ± 3.31	4.1 ± 2.89	0.0466
PRISM	12.39 ± 10.23	4.81 ± 5.67	< 0.0001
PCIS	85.77 ± 7.75	88.75 ± 8.62	0.0405
WBC	8.33 ± 9.17	12.12 ± 30.43	0.4042
NEUT	7.18 ± 7.18 (*n* = 39)	6.41 ± 5.8 (*n* = 66)	0.6677
LY	1.36 ± 1.72 (*n* = 39)	1.69 ± 1.92 (*n* = 66)	0.3990
NEUT%	69.56 ± 22.53 (*n* = 39)	65.39 ± 22 (*n* = 67)	0.4120
LY%	30.53 ± 70.20 (*n* = 39)	24.84 ± 18.12 (*n* = 67)	0.4887
PLT	173.27 ± 161.87	212.32 ± 164.9	0.2195
HBG	111.45 ± 121.47	107.07 ± 20.59	0.7715
BUN	4.93 ± 3.53	4.93 ± 3.79	0.9183
Cr	24.77 ± 12.13	42.61 ± 72.66	0.0975
TBIL	20.09 ± 27.42	12.93 ± 18.34	0.2055
PaO2/FiO2	269.23 ± 135.18	306.73 ± 132.25	0.1618
CRP	67.69 ± 80.16	29.18 ± 46	0.0025
PCT	10.83 ± 27.49	9.82 ± 50.56 (*n* = 67)	0.9259
γ-IFN	5.78 ± 6.24	184.34 ± 987.38 (*n* = 66)	0.2392
IL-6	728.61 ± 1783.29 (*n* = 40)	646.32 ± 2934.9 (*n* = 66)	0.9581
IL-10	360.54 ± 990.91	291.14 ± 1611.67 (*n* = 66)	0.7807
LOHS, mean ± SD	48.7 ± 43.39	16.46 ± 12.11	< 0.0001
ICU, mean ± SD	23.05 ± 31.89	8.72 ± 7.83	0.0005
Mortality rate, *n* (%)	8 (18.18%)	7 (10.14%)	0.2195

[a] Data on clinical indicators were missing for some patients, and "(n=)" indicated the number of people to be tested. BMI: Body Mass Index; pSOFA: Pediatric sequential organ failure assessment; PRISM: pediatric risk of mortality; PCIS: pediatric critical illness score; WBC: white blood cell; NEUT: neutrophil; LY: lymphocyte; NEUT%: neutrophil percentage; LY%: lymphocyte percentage; PLT: platelet; HBG: hemoglobin; BUN: blood urea nitrogen; Cr: creatinine; TBIL: total bilirubin; PaO2/FiO2: the ratio of arterial oxygen partial pressure to fractional inspired oxygen; CRP: C-reactive protein; PCT: procalcitonin; γ-IFN: Interferon-γ; IL-6: Interleukin 6; IL-10: Interleukin 10; LOHS: length of hospital stays; ICU: length of ICU stays

AMRT- group than in AMRT + group (23.19% vs. 6.82%, $P = 0.0233$).

Pathogens detected by CMT and mNGS

The distribution of samples used for mNGS testing is illustrated in Supplementary Fig. 1, with BALF being the most common sample, followed by CSF and blood. The pathogen detection rate in AMRT + samples was significantly higher than that in AMRT- samples, irrespective of the detection method employed (Fig. 1A). Within the AMRT + group, no significant differences were noted in pathogen detection rates across various methods. Conversely, in the AMRT- group, the positive culture rate was markedly lower compared to those of mNGS and CMT ($P < 0.0001$) (Fig. 1A). In the AMRT + Group, the consistency rate (complete match + partial match) between mNGS and culture reached an impressive 75%. However, this proportion was only 1.45% within the AMRT- group (Fig. 1B). When compared to CMT, the consistency of pathogen detection via mNGS was found to be 81.82% in the AMRT + group and merely 21.74% in the AMRT- group (Fig. 1C). Compared to culture and CMT results, the sensitivity of mNGS was 89.74% and 90.70% in AMRT + group, and was 90.01% and 70.59% in AMRT- group (Fig. 1D).

Significant differences were also observed regarding pathogen types detected between both groups. The most common Gram-positive bacteria in both groups were *Streptococcus* and *Staphylococcus*. Gram-positive bacterial detection rates were higher than that of Gram-negative bacteria within the AMRT- group. In contrast, a greater variety of Gram-negative bacteria were

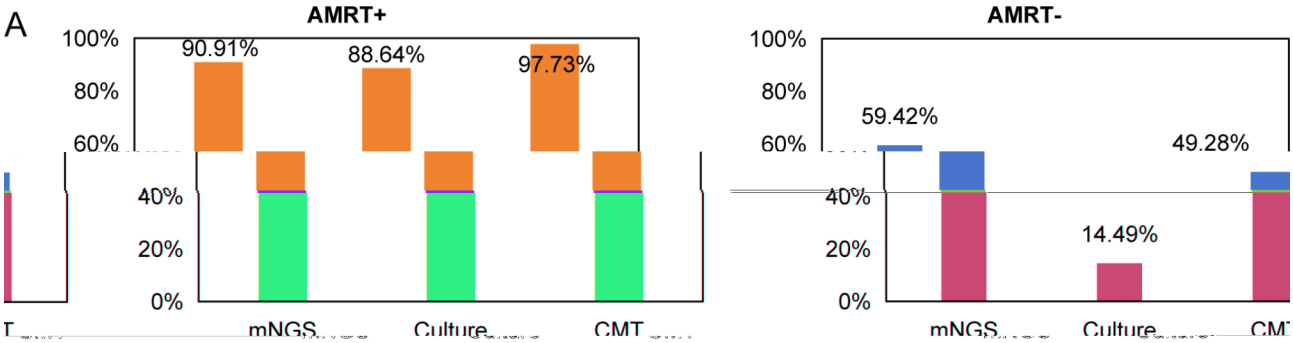
Table 2 Distribution of infection sites and disease types among patients

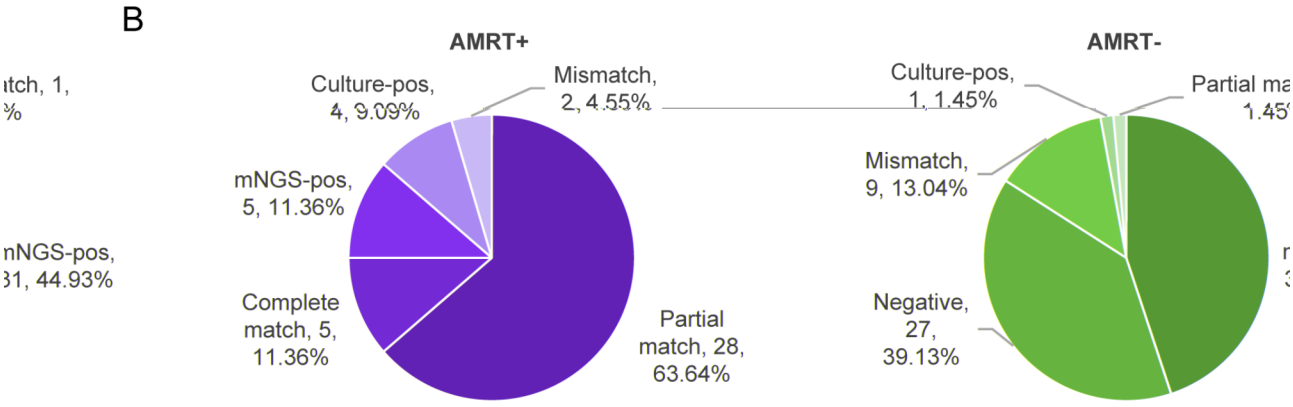
	AMRT+ (*N* = 44) [a]	AMRT- (*N* = 69) [a]	*P*-value
Infection sites, *n* (%)			
Lung	39 (88.64%)	40 (57.97%)	0.0005
Blood	13 (29.55%)	16 (23.19%)	0.4506
Central nervous system	20 (45.45%)	5 (7.25%)	< 0.0001
Abdominal cavity	2 (4.55%)	4 (5.80%)	0.7723
None	6 (13.64%)	0 (0.00%)	0.3243
Liver	1 (2.27%)	0 (0.00%)	0.2084
Gastrointestinal tract	1 (2.27%)	0 (0.00%)	0.2084
Digestive tract	0 (0.00%)	1 (1.45%)	0.4225
Urinary tract	0 (0.00%)	1 (1.45%)	0.4225
Final diagnoses, *n* (%)			
Severe pneumonia	32 (72.73%)	21 (30.43%)	< 0.0001
Sepsis	21 (47.73%)	13 (18.84%)	0.0011
Septic shock	14 (31.82%)	11 (15.94%)	0.0474
Pneumonia	8 (18.18%)	16 (23.19%)	0.5258
Encephalitis	3 (6.82%)	16 (23.19%)	0.0233
Meningitis	2 (4.55%)	4 (5.80%)	0.7723
Tracheitis	3 (6.82%)	1 (1.45%)	0.1321
Pancreatitis	3 (6.82%)	1 (1.45%)	0.1321
Encephalomyelitis	0 (0.00%)	3 (4.35%)	0.161
Liver abscess	1 (2.27%)	1 (1.45%)	0.7462
Enteritis	1 (2.27%)	1 (1.45%)	0.7462
Urinary tract infection	2 (4.55%)	0 (0.00%)	0.074

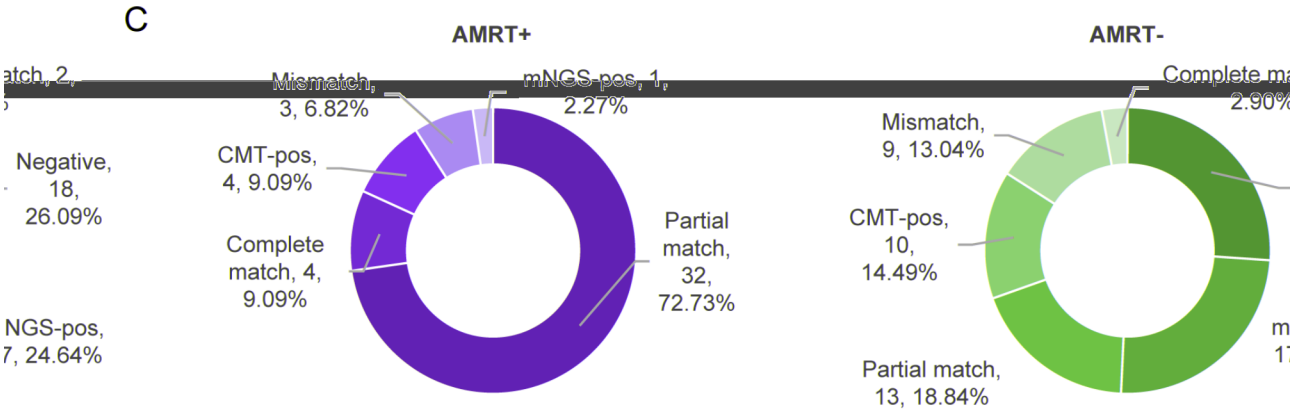
detected in the AMRT+group, mainly *Klebsiella*, *Pseudomonas* and *A. baumannii* (Fig. 2A). *Candida albicans* was the most common detected fungus in both groups, and rare fungi such as *Rhizopus microsporus* and *Cryptococcus neoformans* were also detected in the AMRT+group (Fig. 2B). Human herpesvirus, Human parainfluenza virus and rhinovirus are the most common detected viruses. Notably, more viral types were identified within AMRT- samples compared to those from AMRT+patients (Fig. 2C).

Antibiotics resistance detected by AST and mNGS

The distribution of drug resistance identified in pathogens was systematically summarized and analyzed. Gram-negative bacteria exhibit a broader spectrum of resistance, predominantly resistant to cephalosporins, β-lactams/β-Lactamase inhibitors, carbapenems and sulfonamides (Fig. 3). The resistance profiles of the three most prevalent Gram-negative pathogens to these four classes of antibiotics demonstrated significant variability. *K.pneumoniae* displayed highly levels of resistance to cephalosporins, β-lactams/β-Lactamase inhibitors, and sulfonamides, with rates recorded at 46.15%, 39.29% and 44.44%, respectively; it showed slightly lower resistance to carbapenems at a rate of 21.05%. *P. aeruginosa* exhibited higher resistance rates to carbapenems (33.33%), but lower rates for cephalosporins, β-lactams/β-Lactamase inhibitors and sulfonamides, with rates of 0%, 5.26% and 0%, respectively. *A. baumannii* demonstrated nearly complete resistance to cephalosporins (91.67%), carbapenems (100%) and sulfonamides (100%), alongside a resistance rate of 66.67% against β-lactams/β-Lactamase inhibitors. In contrast, Gram-positive bacteria primarily exhibited resistance towards antibiotics such as penicillins, marcrolides, and lincosamides. Among the antibiotics with sample counts exceeding 22 (50%) in the AST analysis, Levofloxacin, Trimethoprim and sulfamethoxazole, Cefoperazone and Sulbactam, Ceftazidime, Piperacillin and Tazobactam, Imipenem, Cefepime, Tigecycline, Meropenem, Amikacin and Ceftriaxone had a resistance rate of 16.67%, 60%, 33.33%, 46.67%, 41.38%, 41.38%, 37.93%, 0%, 41.67%, 8.70% and 50%, respectively. The drug resistance of pathogens in 2022 and 2023 is highly consistent (Supplementary Fig. 2). Furthermore, we analyzed changes in bacteria resistance to cephalosporins,β-lactams/β-Lactamase inhibitors, carbapenems and sulfonamides over different years, and results showed that *K.pneumoniae* showed lowest resistant to cephalosporins and β-lactams/β-Lactamase inhibitors in 2023; *P. aeruginosa* only showed 14.29% resistance to β-lactams/β-Lactamase inhibitors in 2023, and showed same resistant rate to carbapenems in 2022 and 2023; the resistance rate of *A. baumannii* to carbapenems and sulfonamides was 100% in 2022–2024, and the resistance rate to cephalosporins andβ-lactams/β-Lactamase inhibitors was higher than 50%, with the lowest levels occurring in 2023.







D

CMT	
Pos	Neg
24	17
10	18
70.59%	
51.43%	
58.54%	
64.29%	

AMRT+		Culture	
		Pos	Neg
mNGS	Pos	35	5
	Neg	4	0
Sensitivity		89.74%	
Specificity		0.00%	
PPV		87.50%	
NPV		0.00%	

AMRT+		CMT	
		Pos	Neg
mNGS	Pos	39	1
	Neg	4	0
Sensitivity		90.70%	
Specificity		0.00%	
PPV		97.50%	
NPV		0.00%	

AMRT-		Culture	
		Pos	Neg
mNGS	Pos	10	31
	Neg	1	27
Sensitivity		90.91%	
Specificity		46.55%	
PPV		24.39%	
NPV		96.43%	

AMRT-	
mNGS	Pos
	Neg
Sensitivity	
Specificity	
PPV	
NPV	

Fig. 1 The diagnose performance of mNGS, culture and CMT for pathogens in AMRT + and AMRT- patients. (**A**) The positive rate of mNGS, culture and CMT for pathogens in AMRT + and AMRT- patients. (**B**) The pathogen consistency between mNGS and culture for AMRT + and AMRT- patients. (**C**) The pathogen consistency between mNGS and CMT for AMRT + and AMRT- patients. (**D**) 2 × 2 contingency tables comparing the performance of mNGS relative to clinical testing

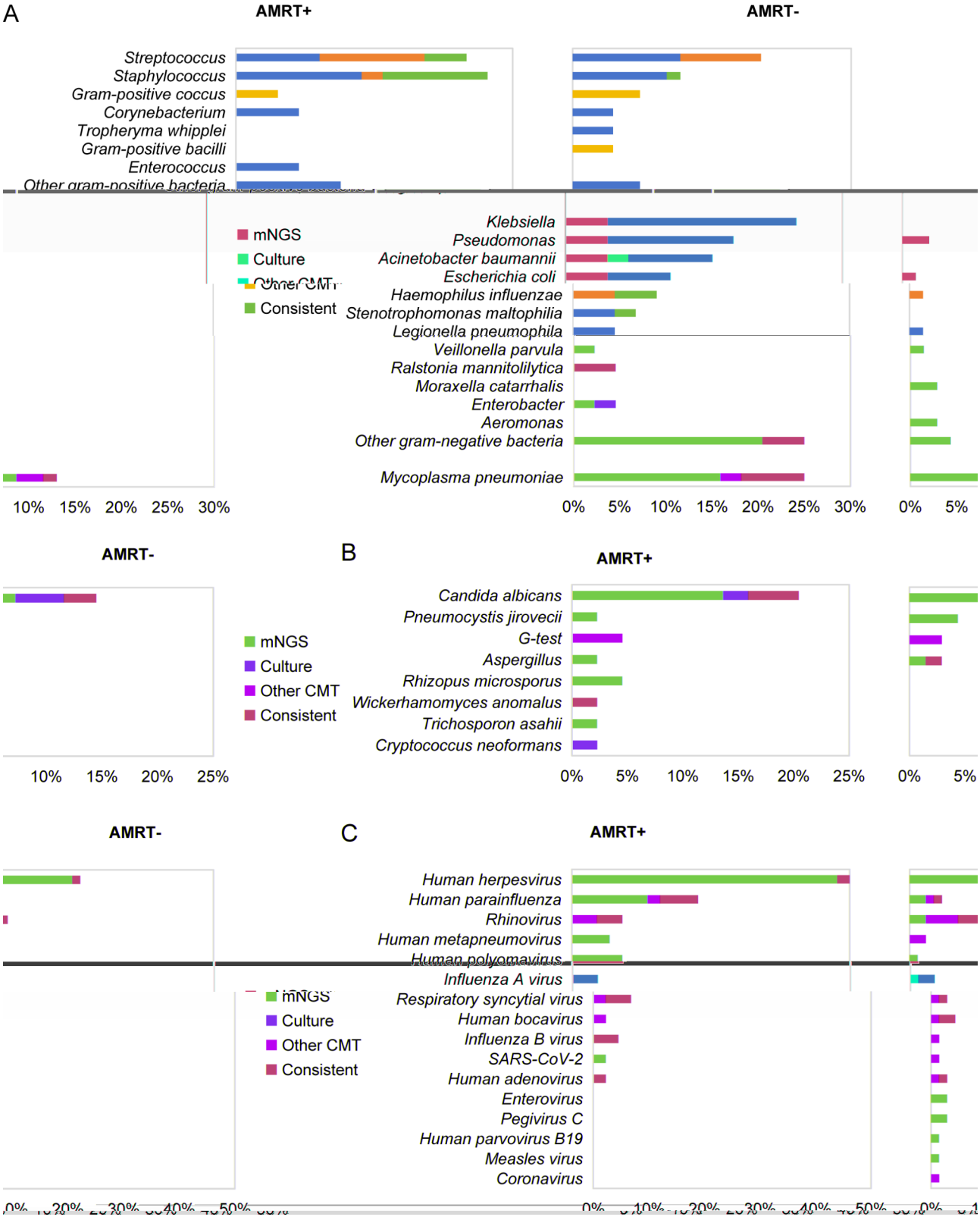


Fig. 2 The pathogen profiles for the AMRT + and AMRT- patients. (**A**) Spectrum of detected bacteria. (**B**) Spectrum of detected fungi. (**C**) Spectrum of detected virus

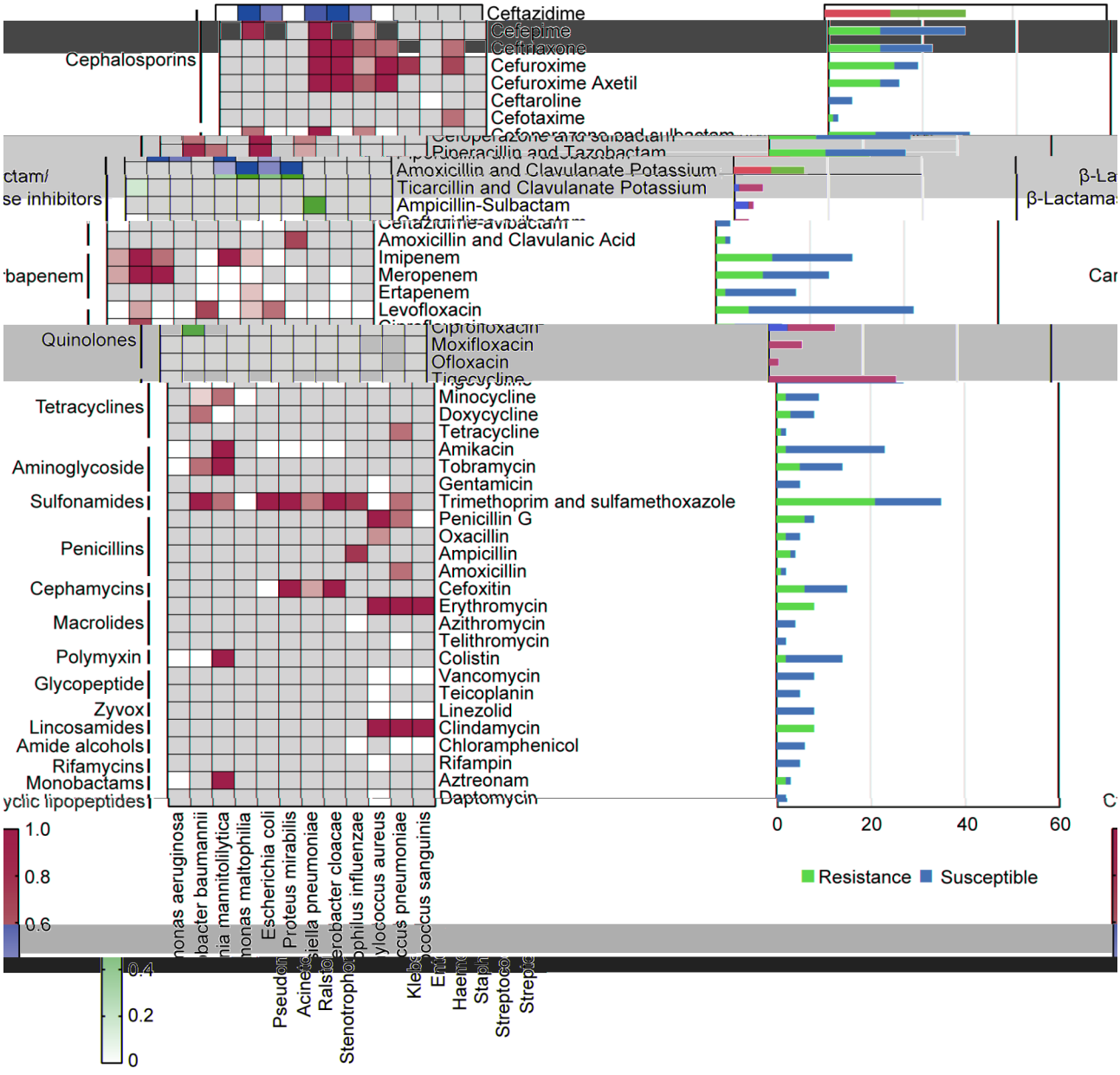


Fig. 3 Antibiotics resistance rate of the AST results. Left panel: heatmap of antibiotics resistance rate by AST; Right panel: AST result for each antibiotic. Gray box means no AST test was performed

A total of 76 drug-resistant genes were detected via mNGS (Supplementary Fig. 4). The most frequently detected gene was *Mex*, followed by *ade*, *mdt*, *OXA*, *Mux*, and *Opm*, which identified for more than 10 times. Using AST results as the gold standard we evaluated mNGS's performance in predicting antibiotic resistances across three classes: cephalosporins, carbapenems and sulfonamides. The sensitivity for predicting carbapenems resistance surpassed that for other categories (66.67% vs. 60.00% vs. 15.00%), and the specificity showed highest value to sulfonamides (92.31%). Furthermore, the accuracy in predicting carbapenems resistance also exceeded that for the other two categories (65.60% vs. 51.28% vs. 45.45%) (Table 3).

Discussion

The emergence of AMR has become a critical issue in the management of infectious diseases, posing significant threats to human health. Microbial identification and AST are two essential tasks conducted by clinical microbiology laboratories to guide the selection of appropriate antimicrobial therapies. In the absence of this information, empirical treatment may result in therapeutic failure or contribute to the rise of antibiotic-resistant pathogens.

Table 3 The performance of mNGS in the prediction of cephalosporins, carbapenems and sulfonamides

Antibiotic	TP	FP	FN	TN	Sensitivity	Specificity	PPV	NPV	Accuracy
cephalosporins	12	11	8	8	60.00%	42.11%	52.17%	50.00%	51.28%
carbapenems	8	7	4	13	66.67%	65.00%	53.33%	76.47%	65.60%
sulfonamides	3	1	17	12	15.00%	92.31%	75.00%	41.38%	45.45%

TN: True negative, TP: True positive, FN: False negative, FP: False positive, PPV: positive predictive value, NPV: negative predictive value. Accuracy=(TP+TN)/(TP+TN+FP+FN)

Monitoring AMR across diverse populations not only aids in guiding the empirical antibiotic use in clinical practice, but also facilitates the development of intervention and prevention strategies [16, 17]. Children are considered a key demographic where antimicrobial drug consumption is high, and they also exhibit a significant prevalence of antimicrobial resistance [18]. A limited understanding of the resistance mechanisms associated with common pediatric pathogens, coupled with a lack of pediatric-specific data, has led to both overuse and misuse of antibiotics, thereby exacerbating antibiotic resistance among pediatric infections-a growing public health concern. Consequently, this study aims to elucidate the distribution patterns of antibiotic resistance among children with infectious diseases within the PICU and clarify the impact of drug-resistant bacteria detection on the prognosis of children.

Antibiotic resistance was observed more frequently in Gram-negative bacterial species than Gram-positive ones, consistent with global observations [19], often leading to MDR [20, 21]. The most common resistant bacteria identified include *Klebsiella*, *Staphylococcus*, *Pseudomonas* and *Acinetobacter*, etc [22]. Our findings corroborated these trends. Notably, the detection rate for Gram-positive bacteria was higher in AMRT- group than that for Gram-negative bacteria. However, an increased prevalence of Gram-negative bacteria was noted within AMRT+group, predominantly *Klebsiella*, *Pseudomonas* and *A. baumannii* (Fig. 2A). These results underscore the necessity for heightened vigilance against emerging antibiotic resistance among children afflicted by Gram-negative bacterial infections.

The review of antibiotic susceptibility patterns for commonly utilized antibiotics, alongside enhanced diagnostic methods for infectious diseases, could serve as effective strategies to mitigate antibiotic resistance. Nevertheless, there is a paucity of studies addressing the distribution of drug resistance among pediatric patients in PICU. Our research, along with a prior investigation on severe pneumonia in children [23], identified *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *K. pneumoniae* as predominant pathogens. The earlier study also examined antimicrobial resistance across 19 antibiotics in pediatric severe pneumonia cases and revealed that pathogen resistance to ceftazidime, meropenem and imipenem exceeded 50% [23]. In our analysis, these Gram-negative bacteria were mainly resistant to cephalosporins, β-lactams/β-Lactamase inhibitors, carbapenems and sulfonamides (Fig. 3). The issue of drug resistance in *A. baumannii* is escalating. Multidrug-resistant *A. baumannii* (MDRAB), extensively drug-resistant *A. baumannii* (XDRAB), and even pandrug resistant *A. baumannii* (PDRAB) isolated from clinical cultures are increasingly prevalent, posing substantial challenges for treatment [24]. Both Gan et al. study [23] and our findings indicated that *A. baumannii* demonstrated high levels of resistance against multiple antibiotics including carbapenems, aminoglycosides, cephalosporins, β-lactams/β-Lactamase inhibitors and sulfonamides. For *P. aeruginosa*, its meropenem resistance rate has shown a decline (from 15.8% in 2016 to 6.1% in 2020) according to the monitoring data from the Chinese Children's Bacterial Resistance Surveillance Group (The Infectious Disease Surveillance of Pediatrics, ISPED) [25]. Nevertheless, in the US pediatric population, the proportion of carbapenem resistant *P. aeruginosa* increased from 9.4% in 1999 to 20% in 2012 [26], and in European pediatric patients, from 2004 to 2012 to 2013–2018, the resistance rate of *P. aeruginosa* to carbapenem antibiotics increased significantly (from 9.1 to 12.4%) [27]. In Gan et al. study, *P. aeruginosa* showed highest resistance towards carbapenems (38.2%) compared to aminoglycosides (0%) and third-generation cephalosporins (17.6%) [23]. In our study, compared to cephalosporins, β-lactams/β-Lactamase inhibitors and sulfonamides, *P. aeruginosa* also showed highest resistance to carbapenems (33.33% vs. 0% vs. 5.26% vs. 0%) (Fig. 3). These findings underscore the necessity for conducting carbapenem resistance testing on suspected resistant strains of *P. aeruginosa* in the PICU. Gan et al. found *K. pneumoniae* with an average resistance rate of 10% to aminoglycosides and 80% against third-generation cephalosporins [23]. Our study found that *K. pneumoniae* exhibited high resistance to cephalosporins, β-lactams/β-Lactamase inhibitors, and sulfonamides, with rates of 48.89%, 39.29% and 44.44%, respectively, while showing relatively less resistant to carbapenems, with a rate of 21.05%.

In addition to the rapidity and high sensitivity of mNGS in pathogen diagnosis, mNGS also holds certain value in the prediction of drug resistance genes. Gan et al. evaluated the predictive capacity of mNGS for cephalosporins, carbapenems, and penicillins, and discovered

that mNGS had the highest sensitivity in predicting carbapenem resistance (67.74%) [23]. Our study obtained same conclusions, compared to AST results, mNGS showed a sensitivity of 66.67% for predicting carbapenems resistance (Table 3). Compared with the diagnostic performance of pathogens, the detection sensitivity of mNGS regarding antibiotic resistance is insufficient. This is due to the fact that there are still certain difficulties in employing mNGS for detecting antibiotic susceptibility. Firstly, there is still a certain gap in the degree of correlation between the reported antibiotic resistance genotype and antibiotic resistance phenotype at present. Secondly, the performance of mNGS in detecting antibiotic resistance genes is influenced by numerous factors, such as the type of pathogen, the type of antibiotic, the sequencing type (DNA or RNA sequencing), and so on. If mNGS is utilized to analyze antibiotic resistant genes in clinical samples, it will demand an extremely high sequencing depth, and the cost will increase by thousands of times. Therefore, the current mNGS cannot fully guide the selection of anti-infective drugs for antibiotic resistant bacteria, and a comprehensive judgment should be made in combination with clinical AST results.

Patients exhibiting compromised health status are more susceptible to developing antibiotic resistance [28]. Consistent with this observation, differences were observed in the clinical characteristics between the AMRT+and AMRT- groups without our study. AMRT+patients had significantly higher percentage of immunosuppression patients (50% vs. 28.99%, $P=0.0242$), or had underlying disease (70.45% vs. 40.58%, $P=0.0019$) when compared to AMRT- patients (Table 1). Moreover, scores used to measure disease severity also showed significantly differences between the two groups, pediatric sequential organ failure assessment (pSOFA) and pediatric risk of mortality (PRISM) scores were significantly higher among AMRT+group whereas pediatric critical illness score (PCIS) was notably greater in the AMRT- group ($P<0.05$). These results suggest that patient's health status is indeed a risk factor for the development of antibiotic resistance. Furthermore, patients infected with drug-resistant bacteria face a higher likelihood of tending to worse clinical outcomes and elevated mortality rates, and consume additional healthcare resources compared to patients infected by non-resistant strains from identical pathogen. In our research, although no significant difference in mortality was detected between the two groups, both length of hospital stay (LOHS) and ICU treatment duration were significantly prolonged in AMRT+group versus AMRT- group (Table 1), indicating that antibiotic resistance also contributes poor prognosis among PICU patients.

While this investigation has attempted to comprehensively analyze the spectrum of drug effectiveness alongside clinical characteristic and impacts on children's prognoses within PICU, this study does possess several limitations. Firstly, it represents a small-scale retrospective analysis therefore conclusions drawn would benefit from further confirmation through larger sample-sized studies. Secondly, pathogen-resistance analyses primarily rely upon clinically derived drug sensitivity results, mNGS serving merely as an auxiliary reference. The distribution of functional genetic markers associated with the resistant phenotype warrants further investigation.

Conclusion

This study analyzed the distribution of antibiotic resistance in children with infectious diseases in PICU and aimed to elucidate the impact of drug-resistant bacteria on the prognosis of children. The findings indicated that immunocompromised children are at a heightened risk for developing resistance. The most common pathogens, including *K. pneumoniae*, *P. aeruginosa* and *A. baumannii*, exhibited distinct resistance profiles; furthermore, the emergence of drug-resistant pathogens is associated with poor prognosis. Gram-negative bacteria demonstrated a greater propensity for acquiring antibiotic resistance. Upon detection of such pathogens, it is imperative to issue early warnings regarding their potential development of resistance to cephalosporins, β-lactams/β-Lactamase inhibitors, carbapenems and sulfonamides.

Abbreviations

AMR	Antimicrobial resistance
PICU	Pediatric intensive care unit
MDRB	Multidrug-resistant bacteria
AST	Antibiotic susceptibility testing
mNGS	Metagenomic next-generation sequencing
ARG	Antibiotic resistance genes
CMT	Conventional microbiological test
CSF	Cerebrospinal fluid
BALF	Bronchoalveolar lavage fluid
PE	Pleural fluid
RPTM	Reads per ten million

Supplementary Information

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Supplementary Material 1

Author contributions

NZ, XZ and YG progressed experimental design, data collection, and manuscript writing and revising. YZ revised the manuscript. WG and ZY contributed to the study conception and design, revised the manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Children's Hospital, Zhejiang University School of Medicine. The data utilized in this study were from retrospective research, the requirement for written informed consent was waived.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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