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Whole genome analysis of *Pantoea* species identified from sepsis patients in selected Ethiopian referral hospitals: emerging pathogens

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Abstract

Background The burden of sepsis worsens due to the continuation of emerging pathogens such as multidrug-resistant *Pantoea* species.

Methods A multicenter study was conducted between October 2019 and September 2020 at four hospitals located in central, southern, and northern parts of Ethiopia. A total of 1416 sepsis patients were recruited and blood cultures were performed. At each study site, positive cultures were characterized by their colony characteristics, gram stain, and conventional biochemical tests. All *Pantoea* species were identified using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI TOF) and subjected to whole genome sequencing (WGS) using Illumina HiSeq 2500. The phylogeny structure of *Pantoea* isolates was calculated using IQ-TREE v1.6.12 from single-nucleotide polymorphisms detected by Snippy v.4.6.0 and filtered by Gubbins v.2.3.4. Average nucleotide identity was estimated by using OrthoANI v.0.93.1 on Shovill v.1.1.0 assemblies. Antimicrobial resistance genes and plasmid replicons were detected using ARIBA v.2.14.6. Phylogenetic trees were visualized using iTOLv.6.5.2.

Results Multiple *Pantoea* species include: *P. dispersa* ($n=19$), *P. septica* ($n=1$), and a novel *Pantoea* spp. ($n=1$), were identified among sepsis patients. All *P. dispersa* isolates and the novel *Pantoea* species were isolated at Dessie Referral Hospital and displayed phylogenetic clonality, including the ubiquity of an IncM1 plasmid and identical antimicrobial resistance (AMR) gene profiles, encoding $bla_{CTX-M-15}$, bla_{TEM-1D}, $bla_{SCO-1,}$ and *aac*(3)-*lla*. The novel *Pantoea* spp. isolate harboured $bla_{CTX-M-9}$ and bla_{TEM-1D} and carried an IncN3 plasmid replicon. The *P. septica* was isolated at Tikur Anbessa Specialized Hospital in Addis Ababa and carried no detectable acquired AMR genes.

Conclusion The emerging *Pantoea* spp. carrying multiple AMR genes were identified from sepsis patients. Implementation of strong infection prevention strategies and building surveillance capacity with advanced bacteriology laboratories capable of identifying multidrug-resistant emerging pathogens is strongly recommended.

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Keywords Sepsis-causing emerging pathogens, Novel *Pantoea* species, Antimicrobial Resistance genes, Plasmids, Whole genome sequencing, Ethiopia

Introduction

Enterobacterales are major aetiologies [1] of sepsis, a life-threatening disorder resulting from a dysregulated immune response to an infection [2]. The Genus *Pantoea* is characterized as flagellated, nonencapsulated, and non-spore-forming *Enterobacterales* [3]. Naturally, it inhabits plants, soil, and water [4] and comprises of several species including *P. agglomerans*, *P. dispersa*, and *P. septica* [3, 5, 6]. *Pantoea* spp. are considered plant pathogens [5] but can cause human infections [7].

Human infections due to *Pantoea* spp. have been reported worldwide [8–10] with *P. agglomerans* and *P. dispersa* as the most common [3, 11]. They can infect various organs and are associated with sepsis, cutaneous infection, acute dacryocystitis, cholangitis, hepatocellular carcinoma, and obstructive pulmonary disease [4, 8–10, 12–17].

In addition, *P. agglomerans* and *P. dispersa* are recognized as emerging sepsis pathogens and reports are increasing [7, 16, 18–20]. *Pantoea* spp. associated sepsis is believed to be due to the contamination of indwelling catheters, intravenous fluids, nutrition supplements, blood products, and their delivery mechanisms [17, 20–23]. Generally, *Pantoea* spp. isolated from human samples are susceptible to most antibiotic classes [23–25], however, the emergence of multidrug-resistant *Pantoea* spp. has been reported [3, 26].

In routine clinical laboratory tests, identification of *Pantoea* spp. using the traditional biochemical methods is difficult due to similarities with other *Enterobacterales* in colony morphology and biochemical reactions [3, 27, 28]. In contrast, the use of advanced bacterial identification systems like MALDI-TOF [29], 16S rRNA, and whole genome sequencing (WGS) enables accurate typing of emerging strains [3, 30, 31].

Globally, there is a scarcity of data related to the magnitude, antimicrobial resistance (AMR) genes, and molecular features of *Pantoea* spp. isolates that cause sepsis. Moreover, in sub-Saharan countries, including Ethiopia, the identification of these emerging pathogens using conventional methods is challenging. This study aimed for whole genome analysis of *Pantoea* species identified from sepsis patients in selected Ethiopian referral hospitals.

Materials and methods

At four teaching/referral hospitals located in central, southern, and northern Ethiopia, a multi-center prospective cross-sectional study was conducted among patients with sepsis between October 2019 and September 2020 (Fig. 1). These hospitals were Tikur Anbessa Specialized Hospital (TASH) and Yekatit 12 Specialized Hospital Medical College (Y12HMC) in central Ethiopia, Hawassa University Comprehensive Specialized Hospital (HUCSH) and Dessie Referral Hospital (DRH) in southern and northern Ethiopia, respectively. Among those who sought medical service in these hospitals, patients suspected of sepsis were included. The attending physician's clinical diagnosis was used to identify eligible patients. All age groups were included, but patients who received antibiotic treatment within the preceding ten days were excluded. The sociodemographic and clinical data of eligible patients were gathered. Data on antibiotics administered for the management of sepsis and patient outcomes were gathered retrospectively. A total of 1416 clinically diagnosed cases of sepsis from different wards were enrolled in this study.

Blood sample collection and transportation

Professional nurses experienced in sample collection for blood culture, and microbiologists at the bacteriology laboratory were recruited as data collectors. Upon patient identification, 1 ml, 2 ml, 3–5, and 5–10 ml of blood was collected from children who were <1, 2–5, 6–11, and 12–17 years old respectively, and 10 ml from adults as shown in the previous publication [32]. For children and adults, a 1:5 and 1:10 blood-to-broth dilution was made respectively. A single blood culture bottle was processed for each patient due to manpower and laboratory setting shortages.

Blood cultures and bacterial identification

All blood culture bottles were incubated aerobically at 37 °C for seven consecutive days and inspected daily for turbidity and hemolysis as signs of bacterial growth. Blood samples that showed growth either before the seventh day of incubation and blood samples that did not show signs of growth on the seventh day were sub-cultured onto blood agar (Oxoid Ltd, UK) and MacConkey agar (Oxoid Ltd, UK) at 37 °C for 24 h. In parallel, a gram stain was performed for each blood sample. All positive cultures were characterized by their colony characteristics, gram staining, and conventional biochemical tests. All bacteria were stored at -70 °C or -16 °C and brought to Sweden for further phenotypic and genomic characterization. All bacteria were stored and phenotypic and genomic analyses were done within one year of isolation.

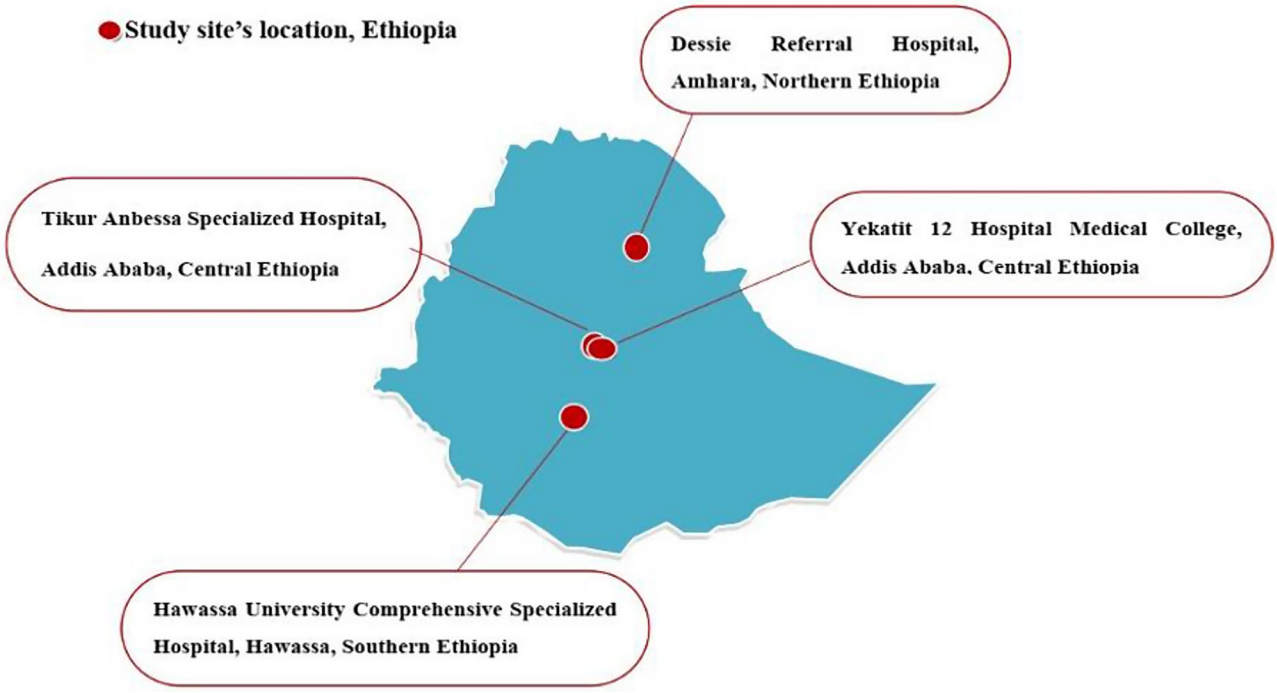

Fig. 1 The four Ethiopian referral hospitals selected for this study and where *Enterobacterales* were isolated

Identification of *P. dispersa*, *P. septica*, and a novel *Pantoea* species

All bacteria were reidentified using MALDI-TOF, as described in the previous publication [32], at the Clinical Microbiology Department of Uppsala University Hospital and Karolinska Institute, Sweden. WGS was subsequently performed on all *Pantoea* isolates for confirmation, utilizing the raw sequence reads and assembled genomes.

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing (AST) was performed using disk diffusion and interpreted based on the standardized table supplied by the Clinical and Laboratory Standards Institute [33] as described in the previous work [32]. Initially, the AST was performed at each study site and re-performed at Uppsala University.

DNA extraction and whole genome sequencing (WGS)

DNA was extracted from all *Pantoea* isolates using QIAamp DNA Mini Kit (QIAGEN, Germany) by taking 2–5 pure colonies that grew on cystine lactose electrolyte deficient agar at 37 °C for 24 h aerobically. DNA concentrations were measured with QubitTM3.0 (Thermo-Scientific, MA, USA). All DNA samples were submitted for WGS at the Science for Life Laboratory, Solna, Sweden. WGS was performed using Illumina HiSeq 2500 systems and genome assembly was done using SPAdes (version 3.9).

Bioinformatic quality control

Illumina sequencing reads were assessed for quality using FastQC v.0.11.5 [34] and trimmed for adapter sequences and quality control by Trimmomatic v.0.39 [35], with a sliding window quality cut-off value of Q20. Assembly quality was assessed using Quast v.5.0.2 [36]. All assemblies yielded less than 100 contigs and the mean total coverage ranged from 84x to 136x.

Antimicrobial resistance gene and plasmid replicon prediction

AMR gene presence was predicted using ARIBA v2.14.6 [37] by querying against the SRST2-ARGANNOT [38] database. Similarly, using ARIBA plasmid replicon typing was performed by querying against the PlasmidFinder database [39]. Gene and replicon identity was verified by extracting their respective sequences from *de novo* Shovill v.1.1.0 [40] assemblies annotated by Prokka v1.14.6 [41] via Artemis v.16.0.17 [42]. These sequences were then compared against their respective reference sequences from the European Nucleotide Archive using Clustal Omega [43].

Phylogenetic analysis

A core genome tree of *Pantoea* spp. isolates was constructed by mapping sequencing reads against the reference *P. dispersa* genome (ASM1989095v1) using Snippy v.4.6.0 [44]. One *P. septica* (SRR5329931), *P. wallisii* (ASM209548v1) and *P. agglomerans* (DRR294260) isolate were also included as outgroups. Recombinant

regions were then excluded from the alignment by Gubbins v.2.3.4 [45]. ACTG-only SNPs were extracted from the recombination-free alignment using snp-sites v.2.3.3 [46]. Tree calculation was performed using IQ-TREE v.1.6.12 [47], with the general time reversible model and gamma correction using ascertainment bias correction (-m GTR+G+ASC) at 1000 bootstrap replicates.

A condensed core genome tree was similarly constructed to display the Average Nucleotide Identity between representative strains, calculated using OrthoANI v.0.93.1 [48] on the Shovill assemblies. The resultant consensus trees were annotated using the Interactive Tree of Life [49]. Genome accession numbers, sequencing and assembly quality statistics, and coding workflows are available in the Supplementary material (S1 and S2).

Assembly and BLAST analysis for species identification

Using the assembled genome, the Basic Local Alignment (BLAST) search for *Pantoea* isolates was done using the BLASTN version 2.13.0+tool at the National Center for Biotechnology Information (NCBI).

Table 1 Frequency of *Pantoea* species in relation to patient sociodemographic characteristics

Patient characteristics		Frequency of *Pantoea* species isolated (*n*=21)	
		No.	Percentage (%)
Hospital	DRH	20	95
	TASH	1	5
Gender	Male	11	52
	Female	10	48
Age category	≤29 days	18	86
	30 days – ≤5 year	-	-
	>5 - <18 year	1	5
	≥18 years	2	10
Ward	NICU	18	85
	EOPD	2	10
	Medical Ward	1	5
Hospital stay duration	1 week	19	90
	2 weeks	1	5
	3 weeks and above	1	5
Underlying diseases	Yes	1	5
	No	20	95
Previous hospitalization	Yes	2	10
	No	19	90
Referral patient*	Yes	4	19
	No	17	81

TASH – Tikur Anbessa Specialized Hospital; Y12HMC – Yekatit 12 Specialized Hospital Medical College; DRH – Dessie Referral Hospital, HUCSH – Hawassa University Comprehensive Specialized Hospital; * Patients who were transferred from other healthcare facilities to the study sites

Average nucleotide identity (ANI) determination

The novelty of a *Pantoea* species was assessed using Average nucleotide Identity (ANI) determination. ANI was calculated using an EZBioCloud online tool [50].

Statistical analysis

The data was analysed using SPSS version 28 for analysis. Descriptive statistics (count, mean, percentages or frequency, and standard deviation) were calculated. The frequencies of acquired AMR genes and plasmid replicons were calculated. Chi-square was used to determine associations of sociodemographics with *Pantoea* species positivity rate. A p-value<0.05 was considered statistically significant.

Results

Pantoea species: identification, frequencies and whole genome sequencing

In this study, a total of 1416 patients clinically investigated for sepsis were enrolled (as described in the previous work [32], and 21 *Pantoea* isolates were identified. While 20 *Pantoea* isolates were identified at DRH from the north, only one strain was isolated at TASH from central Ethiopia (Table 1). No *Pantoea* species were isolated at Y12HMC from central and at HUCSH in the southern parts of the country. The majority of *Pantoea* isolates (*n*=18) were identified at the neonatal intensive care unit (NICU) of DRH from patients of age ≤29 days (Table 1). While two *Pantoea* isolates were identified from adult patients at the emergency outpatient department (EOPD) of DRH, one *Pantoea* isolate was isolated at the medical ward of TASH (Table 1).

Initially, all *Pantoea* isolates were incorrectly classified as *Serratia* or *Acinetobacter* species using conventional methods. All *Pantoea* isolates were reclassified as *P. dispersa* using MALDI-TOF. Furthermore, all *P. dispersa* were reidentified using sequence-based core genome and ANI analysis. From the 21 putative *P. dispersa* isolates, one was retyped as *P. septica* and another as a novel *Pantoea* species that was different from but most closely related to *P. agglomerans.* The rest were classified as *P. dispersa* (*n*=19).

Clinical characteristics and patient outcomes

All patients whose blood cultures were positive for *Pantoea* species had a fever (>38 °C) (Table 2). The majority of these patients had elevated respiratory rate, heart rate, and high or low white blood cell count. Among 18 neonates, 16 of them were diagnosed with early-onset neonatal sepsis (EONS) and 2 with late-onset neonatal sepsis (LONS). While the majority of patients had no underlying disease, one patient had acute lymphoid leukaemia (S3). Of 20 septic patients with *Pantoea* species at DRH,

Table 2 Antimicrobial susceptibility patterns of *Pantoea species* identified from sepsis patients in Ethiopian referral hospitals

Patient ID	Pantoea species	AMK	AMP	AMC	SAM	ATM	FEP	CTX	CRO	CAZ	CXM	CIP	C	DO	GEN	MEM	TZM	SXT	TE
194	*P. dispersa*	S	R	I	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S
195	*P. dispersa*	S	R	I	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S
219	*P. dispersa*	S	R	R	I	R	R	R	R	R	R	S	S	S	R	S	S	S	S
221	*P. dispersa*	S	R	R	S	R	R	R	R	R	R	S	S	S	R	S	S	S	S
222	*P. dispersa*	S	R	I	R	R	R	R	R	R	R	S	S	S	R	S	R	S	S
237	*P. dispersa*	S	R	I	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S
243	*P. dispersa*	S	R	I	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S
265	*P. dispersa*	S	R	I	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S
262	*P. dispersa*	S	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S
268	*P. dispersa*	R	R	R	R	R	R	R	R	R	R	S	S	S	R	S	R	S	S
266	*P. dispersa*	I	R	I	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S
239	*P. dispersa*	S	R	I	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S
275	*P. dispersa*	S	R	I	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S
277	*P. dispersa*	S	R	I	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S
288	*P. dispersa*	S	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S
291	*P. dispersa*	S	R	I	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S
297	*P. dispersa*	S	R	I	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S
305	*P. dispersa*	S	R	I	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S
319	*P. dispersa*	S	R	S	S	R	I	R	R	I	R	S	R	R	S	S	S	R	R
142	*P. septica*	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
218	Novel *Pantoea* species	S	R	R	S	R	I	R	R	S	R	S	S	S	S	S	S	S	S

R – resistance; I – intermediate; AMK –amikacin; AMP – ampicillin; AMC – amoxicillin/clavulanate; SAM – ampicillin-sulbactam; ATM – aztreonam; FEP – cefepime; CTX – cefotaxime; CRO – ceftriaxone; CAZ – ceftazidime; CXM – cefuroxime; CIP – ciprofloxacin; C – chloramphenicol; DO – doxycycline; GEN – gentamicin; MEM – meropenem; TZP – piperacillin/tazobactam; SXT – trimethoprim-sulfamethoxazole; TE – tetracycline

4 of them had been referred to the hospital from other health facilities.

Patient outcomes were gathered for 18 patients retrospectively. On the same day after blood sample collection for blood culture, patients were treated with either ampicillin and gentamicin or ampicillin and cefotaxime (S4). Of 18 patients who were diagnosed with EONS, LONS, or sepsis and whose blood culture gave positive results for *Pantoea* species, 12 of them showed improvement while 5 patients died (Patient ID 194, 237, 265, 277, and 291) (S4). In one case, the patient was registered as self-discharge against medical advice (S4). The status of three patients (Patient ID 142, 195, and 221) could not be determined because their clinical charts had been lost for an unknown reason.

Antimicrobial susceptibility patterns of *Pantoea* species

Almost all *Pantoea* isolates were resistant to ampicillin, aztreonam, cefotaxime, and ceftriaxone that were prescribed for patients as first-line or second-line regimens (Table 2). While one *P. dispersa* (Sample ID 319) was sensitive to ceftazidime, cefuroxime, and gentamicin, all other *Pantoea* species were resistant (Table 2). The novel *Pantoea* spp. isolate showed resistance against ampicillin, amoxicillin-clavulanic acid, aztreonam, cefotaxime, ceftriaxone, ceftazidime, and cefuroxime (Table 2).

Genetic diversity and phylogenetic structure of *Pantoea* isolates

Among the *Pantoea* spp., *P. dispersa* ($n=19$), *P. septica* ($n=1$), and a novel *Pantoea* spp. were identified using a core genome maximum likelihood tree (Fig. 2). All *P. dispersa* isolates displayed clonality in the tree, suggesting a clonal outbreak in the hospital, which was further supported by ANI analysis (Fig. 3). While 17 *P. dispersa* were isolated in the NICU, two others were identified in EOPD of DRH (Sample ID 222 and 305). Among the 4 septic patients who had a referral history at DRH, clonally related *P. dispersa* were identified among 3 of them who were referred from government health institutions while the novel *Pantoea* species was identified from a patient sent from a private health facility.

The *P. dispersa* isolates were, as expected, most closely related to the *P. dispersa* reference genome. The two most distantly related *P. dispersa* were also 99.5% identical. The *P. septica* strain, isolated in TASH, grouped with *P. septica* within the conventional 95% ANI species cut-off (Fig. 3).

The novel *Pantoea* spp. was initially identified as *P. dispersa* by MALDI-TOF, but it most closely grouped with the *P. agglomerans* outgroup. However, it was too distantly related in the core genome tree to be considered *P. agglomerans*. This was confirmed by ANI analysis citing 84.52% identity; thus, its species remains unconfirmed. The isolates of *Pantoea* spp. were collected at different time points over the collection period with a majority

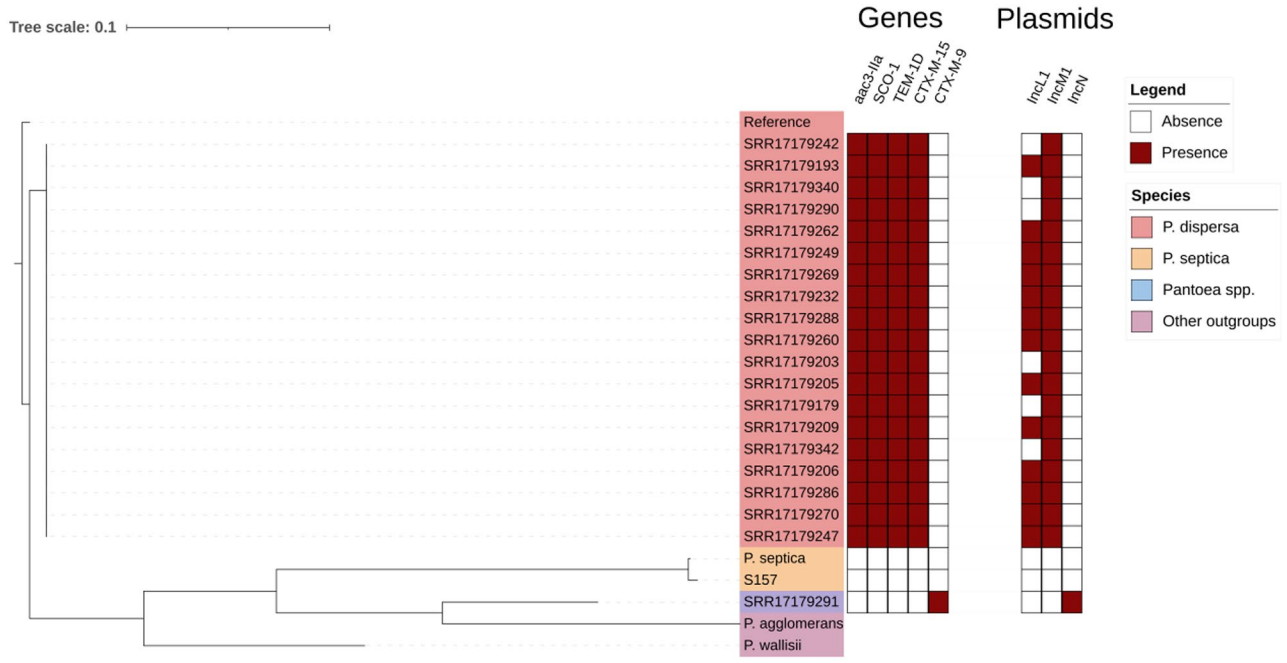

Fig. 2 Maximum likelihood tree generated from the core genome of 21 *Pantoea* isolates identified from sepsis patients in Ethiopian referral hospitals, alongside Pantoea spp. representative outgroups

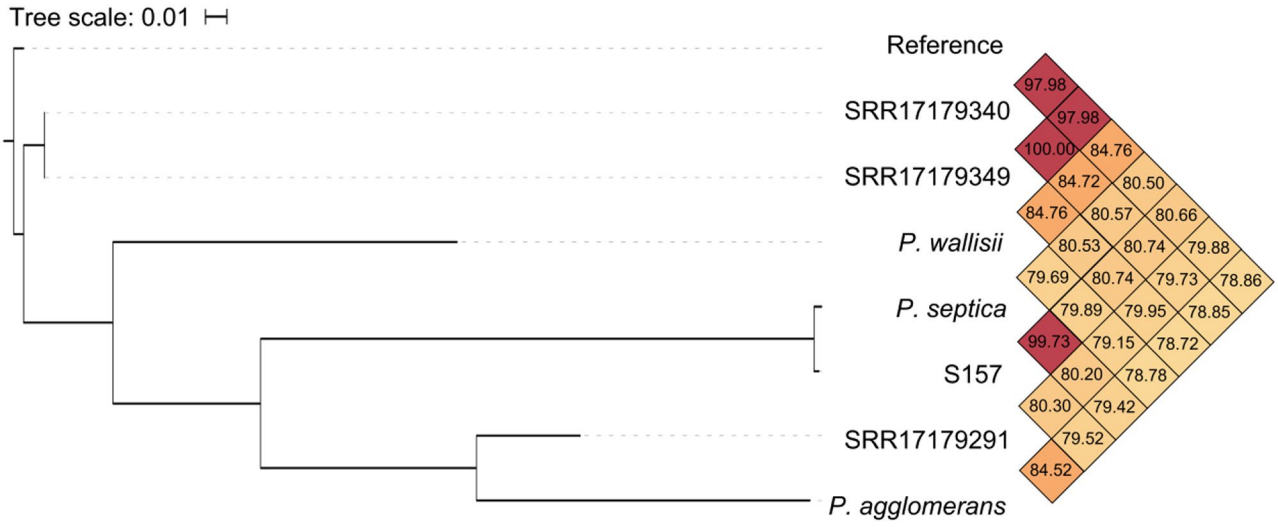

Fig. 3 Average Nucleotide Identity matrix against a condensed maximum likelihood core genome tree. The two most distantly related *P. dispersa* isolates and the *P. septica* and novel *Pantoea spp*. strains were included alongside *Pantoea. spp*. outgroups

within a short period of time towards the end of the collection time in April and May 2020 (see details in S4).

Antimicrobial resistance genes and plasmid replicons of *Pantoea* isolates

All *P. dispersa* were predicted to have identical AMR gene profiles, containing the β-lactamase genes $bla_{CTX-M-15}$, $bla_{SCO-1,}bla_{TEM-1D,}$ and the aminoglycoside resistance gene *aac*(3)-*IIa* (Fig. 2). However, ARIBA calculated 99.88% identity to the bla_{TEM-1D} reference sequence and alignment against this reference revealed a 2 bp variation. In contrast, the novel *Pantoea* spp. was predicted to contain only $bla_{CTX-M-9}$ while the *P. septica* isolate was devoid of acquired AMR genes (Fig. 2).

The *P. dispersa* isolates were predicted to ubiquitously carry the IncM1 plasmid replicon while 68.4% (n=13/19) also possessed IncL1 (Fig. 2). The most similar plasmid with IncM1 in the GenBank was *Klebsiella pneumoniae* plasmid pMRY13-133KPN_2 (GenBank accession number AP018454.1). All AMR genes carried by *P. dispersa* were located on the same 80 kb contig as the IncM1 replicon gene was located. The novel *Pantoea* spp. carried neither, instead carrying IncN3 located in the same 38.7 kb contig as $bla_{CTX-M-9}$. The most similar plasmid to IncN3 in GenBank was *pC52_003* from *Klebsiella michiganensis* strain C52 (GenBank accession number CP042548.1). No plasmid replicons were detected within the *P. septica* isolate (Fig. 2).

Discussion

The World Health Organization has recognized sepsis as a major public health problem, especially in low and middle-income countries [51]. The burden of sepsis worsens due to emerging pathogens that are challenging for accurate identification [52] and clinical management due to increasing multidrug resistance [11]. Human infections of *Pantoea* spp. have been reported as uncommon [22]. However, recently it is considered an emerging human pathogen [53]. The current study showed the emergence of *Pantoea* spp. infection among patients investigated for sepsis in Ethiopia, and a likely local clonal outbreak of *P. dispersa*.

In the current study, among a total of 1416 patients clinically investigated for sepsis from four Ethiopian referral hospitals 21 cases of *Pantoea* were identified at two hospitals. *Pantoea* spp. isolates (n=20) were identified at DRH in the north and one case at TASH in central Ethiopia. Among the 21 sepsis cases due to *Pantoea* spp., 18 were identified at the NICU of DRH that showed similarities with other cases of neonatal sepsis due to *Pantoea* spp. worldwide [16, 22, 54]. Of 18 cases of sepsis due to *Pantoea* spp, 5 neonates died at the NICU of DRH. Their main cause of death may not be determined exactly due to the cross-sectional nature of the study design. However, it could be associated with the sepsis caused by *Pantoea* strains which were resistant to first-line and second-line antibiotic regimens administered for the treatment of neonatal sepsis.

The findings of the current study showed that *Pantoea* spp. are emerging as human pathogens causing sepsis. Moreover, the identification of *Pantoea* isolates more frequently at DRH calls for site-specific infection prevention strategies. *Pantoea* spp. associated neonatal sepsis in NICU may be due to contamination of parenteral nutrition, infant formula, intravenous fluid, blood products, and anaesthetic agents [22, 23, 54]. However, the source of *Pantoea* isolates for the current study was not identified. A follow-up investigation is necessary to explain

whether the identification of *Pantoea* spp. isolates at DRH was a temporary outbreak or has persisted.

Using the conventional method, all *Pantoea* spp. isolates were misidentified as *Serratia* or *Acinetobacter* but typed accurately as *Pantoea* spp. using MALDI-TOF. However, after WGS determination, all were identified as *P. dispersa* except for two isolates: a *P. septica* and a novel *Pantoea* spp. isolate. Misidentification of *Pantoea* spp. using the conventional method has been reported [3, 27] and could be due to the shared colony and biochemical characteristics with other *Enterobacterales.* The inaccurate classification of a few *Pantoea* isolates as *Acinetobacter* species showed professional skill gaps in identifying *Pantoea* species. Considering the identification of *Pantoea* species, the implementation of advanced bacterial identification techniques in routine laboratory checks is necessary. However, the availability of resources could be the key determinant for microbiology laboratories in low-resource settings. Expansion of accurately identified species to include emerging pathogens such as *Pantoea* spp. would benefit healthcare systems, allowing them to tailor treatment and prevention programs.

P. dispersa isolates were phylogenetically identical and circulated both in the NICU or EOPD wards of DRH. Identification of *P. dispersa* among sepsis patients has been reported by other studies [3, 4, 15, 22, 55], however, this showed a possible clonal outbreak in Ethiopia. The identification of clonally related *P. dispersa* among patients referred from other governmental health institutions could suggest that these strains can be rapidly acquired in the hospital. The identification of a novel *Pantoea* isolate at DRH showed that other *Pantoea* spp. could emerge in clinical settings. Furthermore, since the novel *Pantoea* species was isolated from a patient referred from a private health facility the strain could be carried to the hospital from other sources. More importantly, the identification of *P. septica* in another hospital (TASH) located in a different region (Central Ethiopia) shows that, although rare, *Pantoea* spp. infections were not regionally locked and emerged unrelated to the *P. dispersa* clonal outbreak at DRH.

All *P. dispersa* encoded $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1D}}$, $bla_{\text{SCO-1}}$, and *aac*(3)-*lla* and a similar plasmid of type IncM1 that was associated with the acquired AMR genes. This study showed the emergence of *P. dispersa* with a plasmid-encoded ESBL gene from Ethiopia however $bla_{\text{CTX-M-15}}$ carried by *P. agglomerans* was reported from Nigeria [11]. The novel *Pantoea* spp. isolate harbored $bla_{\text{CTX-M-9}}$ and $bla_{\text{TEM-1D}}$ and carried the plasmid replicon IncN linked with $bla_{\text{CTX-M-9}}$. The identification of *Pantoea* spp. that carried plasmid-mediated, and multiple, AMR genes calls for the implementation of robust antimicrobial stewardship and within-hospital microbiological monitoring and surveillance. The use of whole genome sequencing to determine the identity of the bacteria isolated from sepsis patients is a strength of this study and has revealed an unusual occurrence of multi-drug-resistant *Pantoea* spp.

Limitations of the study

At study sites, the conventional microbiology methodology was unable to accurately identify *Pantoea* species, and the lab researchers were not alerted to sample additional *Pantoea* isolates and search for any sources of contamination. Though it is rare to identify gram-negative bacteria as blood culture contaminants [56], the possibility can not be ruled out. However, it could be noted that the *Pantoea* isolates were identified from different patients on several occasions throughout the year. Limitations of the study also include the time taken to carry out correct identification as this precluded any effort for real-time follow-up for the patients in question. More refined and robust clinical microbiology is required to accurately identify bacterial isolates within a useful timescale in terms of patient benefit.

Conclusion

Pantoea isolates were identified as emerging pathogens among sepsis patients in Ethiopian referral hospitals which calls for better infection prevention and control strategies. The emergence of *Pantoea* isolates encoding plasmid-linked AMR genes needs special emphasis and strong antimicrobial stewardship. Since *Pantoea* isolates were misidentified using conventional methods, the implementation of advanced bacterial identification techniques is necessary.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-024-03561-5.

Supplementary Material 1: S1 shows the coding workflow for the whole genome analysis of *Pantoea* species identified from sepsis patients in selected Ethiopian referral hospitals: emerging pathogens. S2 is a table that shows the Genome accession number, sequencing, and assembly quality statistics. S3 shows the clinical characteristics of patients with positive blood cultures for *Pantoea* isolates. S4 shows the antibiotics prescribed and clinical outcomes of patients whose blood cultures were positive for *Pantoea* species.

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Author contributions
MHL: Conceptualization, Methodology, Investigation, Software, Data curation, Formal analysis and writing—original manuscript. DA: supervision and writing—review and editing. RP: software, formal analysis and writing—review and editing. BH: methodology and writing—review and editing. AM: conceptualization, funding acquisition, resources, project administration, supervision and writing—review and editing. AA: conceptualization, methodology, supervision and writing—review and editing. AR: software, formal analysis, and writing—review and editing. GS: conceptualization, methodology, software, data curation, funding acquisition, resources, project administration, supervision, and writing—review and editing. All authors read and approved the final manuscript.

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Data availability
The datasets generated and/or analysed during the current study are available in the National Center for Biotechnology Information repository (BioProject ID: PRJNA787062) https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA787062.

Declarations

Ethics approval and consent to participate
The study was approved by the Department of Microbiology, Immunology and Parasitology Ethical Review Committee (DEREC/18/19/01-H) and Institutional Review Board (AAUMF 01–008) of the College of Health Sciences, Addis Ababa University. The study was also approved by the AHRI/ALERT Ethics Review Committee (protocol number: P050/18) of the Armauer Hansen Research Institute and National Ethical Review Committee (Ref No. MoSHE//RD/14.1/690/19). Written informed consent was obtained from all study participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Amanati A, Sajedianfard S, Khajeh S, Ghasempour S, Mehrangiz S, Nematolahi S, et al. Bloodstream infections in adult patients with malignancy, epidemiology, microbiology, and risk factors associated with mortality and multi-drug resistance. BMC Infect Dis. 2021;21(1):1–14.
2. WHO. Global report on the epidemiology and burden of sepsis: current evidence, identifying gaps and future directions. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO. https://www.who.int/publications/i/item/9789240010789
3. Yang Y, Hu H, Zhou C, Zhang W, Yu Y, Liu Q, et al. Characteristics and accurate identification of Pantoea dispersa with a case of spontaneous rupture of hepatocellular carcinoma in China: a case report. Medicine. 2022;101(2):e28541.
4. Mehar V, Yadav D, Sanghvi J, Gupta N, Singh K. Pantoea dispersa: an unusual cause of neonatal sepsis. Brazilian J Infect Dis. 2013;17(6):726–8.
5. Brady C, Cleenwerck I, Venter S, Vancanneyt M, Swings J, Coutinho T. Phylogeny and identification of Pantoea species associated with plants, humans and the natural environment based on multilocus sequence analysis (MLSA). Syst Appl Microbiol. 2008;31(6–8):447–60.
6. Lo CI, Padhmanabhan R, Mediannikov O, Nguyen TT, Raoult D, Fournier PE, et al. Genome sequence and description of Pantoea septica strain FF5. Stand Genomic Sci. 2015;10:103.
7. Van Rostenberghe H, Noraida R, Wan Pauzi W, Habsah H, Zeehaida M, Rosliza A, et al. The clinical picture of neonatal infection with Pantoea species. Jpn J Infect Dis. 2006;59(2):120.
8. Zuberbuhler B, Carifi G, Leatherbarrow B. Acute Dacryocystitis in a 2-Year Old Child caused by Pantoea. Orbit. 2012;31(1):13–4.
9. Im JH, Yeo JY, Park SW, Yang DH, Kim MS, Kim JJ, et al. A case of Pantoea species Cholangitis with Bacteremia. Soonchunhyang Med Sci. 2012;18(2):148–50.
10. Okwundu N, Mercer J. *Pantoea agglomerans* cutaneous infection. J Dermatology Dermatol Surg. 2019;23(1):41–3.
11. Aibinu I, Pfeifer Y, Peters F, Ogunsola F, Adenipekun E, Odugbemi T, et al. Emergence of bla CTX-M-15, qnrB1 and aac(6')-Ib-cr resistance genes in Pantoea agglomerans and Enterobacter cloacae from Nigeria (sub-saharan Africa). J Med Microbiol. 2012;61(1):165–7.
12. Shrestha B, K C N, Bastola C, Jahir T, Risal R, Thapa S, et al. Pantoea agglomerans: an elusive contributor to Chronic Obstructive Pulmonary Disease Exacerbation. Cureus. 2021;13(10):e18562.
13. Pandya S, Aslam S, Shenoy R, Denham JD, Nanjappa S, Greene JN. Pantoea infections in Cancer patients: a Retrospective Chart Review and Review of Literature. Infect Dis Clin Pract. 2018;26(5):275–8.
14. Gan HM, Parthasarathy A, Henry KR, Savka MA, Thomas BN, Hudson AO. Whole-genome sequencing of < i > Pantoea sp. Strain RIT388, a potential oral opportunistic Pathogen isolated from a chewing Stick (< i > Distemonanthus benthamianus). Microbiol Resource Announcements. 2020;9(9):e01468–19.
15. Asai N, Koizumi Y, Yamada A, Sakanashi D, Watanabe H, Kato H, et al. Pantoea dispersa bacteremia in an immunocompetent patient: a case report and review of the literature. J Med Case Rep. 2019;13(33):1–5.
16. Sengupta M, Banerjee S, Das NK, Guchhait P, Misra S. Early Onset neonatal septicaemia caused by Pantoea agglomerans. J Clin Diagn Res. 2016;10(5):Dd01–2.
17. Siwakoti S, Sah R, Rajbhandari RS, Khanal B. *Pantoea agglomerans* Ininfectionsn ChchildrenRereportf Twtwoases. Case Rep Pediatr. 2018;2018:1–3.
18. Tiwari S, Beriha SS. Pantoea species causing early onset neonatal sepsis: a case report. J Med Case Rep. 2015;9(188):1–3.
19. Yablon BR, Dantes R, Tsai V, Lim R, Moulton-Meissner H, Arduino M, et al. Outbreak of < i > Pantoea agglomerans bloodstream infections at an Oncology Clinic—Illinois, 2012–2013. Infect Control Hosp Epidemiol. 2017;38(3):314–9.
20. Oliveira MI, Batalha S, Gouveia C, Maia R, Kjöllerstrom P. Pantoea species Bacteremia in a child with Sickle Cell Disease: looking for a culprit. J Pediatr Hematol Oncol. 2017;39(6):e307–8.
21. Schmid H, Weber SSC, Bogner JR. Isolation of a Pantoea dispersa -like strain from a 71-Year-old woman with Acute myeloid leukemia and multiple myeloma. Infection. 2003;31(1):66–7.
22. Mani S, Nair J. Pantoea infections in the neonatal Intensive Care Unit. Cureus. 2021;13(2):e13103.
23. Mardaneh J, Dallal MM. Isolation, identification and antimicrobial susceptibility of Pantoea (Enterobacter) agglomerans isolated from consumed powdered infant formula milk (PIF) in NICU ward: first report from Iran. Iran J Microbiol. 2013;5(3):263–7.
24. Kaur IP, Inkollu S, Prakash A, Gandhi H, Mughal MS, Du D. Pantoea agglomerans Bacteremia: is it dangerous? Case Rep Infect Dis. 2020;2020:7890305.
25. Gajdács M. Epidemiology and antibiotic resistance trends of Pantoea species in a tertiary-care teaching hospital: a 12-year retrospective study. Developments Health Sci. 2019;2(3):72–5.
26. AbdAlhussen LS, Darweesh MF. Prevelance and antibiotic susceptibility patterns of Pantoea spp. isolated form clinical and environmental sources in Iraq. Int J ChemTech Res. 2016;9(8):430–7.
27. Bhatti MD, Kalia A, Sahasrabhojane P, Kim J, Greenberg DE, Shelburne SA. Identification and whole genome sequencing of the first case of Kosakonia

radicincitans causing a human bloodstream infection. Front Microbiol. 2017;8:62.
28. Sng ECY, Goh KCM, Tan SH, Tan AL, Oh HML. Leclercia adecarboxylata bacteraemia: clinical features and antibiotic susceptibilities in 2 hospitals in Singapore. Ann Acad Med Singapore. 2021;50(8):643–5.
29. Singhal N, Kumar M, Kanaujia PK, Virdi JS. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. Front Microbiol. 2015;6:791.
30. Soutar CD, Stavrinides J. Molecular validation of clinical Pantoea isolates identified by MALDI-TOF. PLoS ONE. 2019;14(11):e0224731.
31. Zayet S, Lang S, Garnier P, Pierron A, Plantin J, Toko L, et al. Leclercia adecarboxylata as Emerging Pathogen in Human infections: clinical features and Antimicrobial susceptibility testing. Pathogens. 2021;10(11):1399.
32. Legese MH, Asrat D, Swedberg G, Hasan B, Mekasha A, Getahun T, et al. Sepsis: emerging pathogens and antimicrobial resistance in Ethiopian referral hospitals. Antimicrob Resist Infect Control. 2022;11(83):1–6.
33. Institute CLS. Performance standards for antimicrobial susceptibility testing;30th ed. CLSI. 2020;M100.
34. Andrews S. FastQC: A Quality Control Tool for High Throughput Sequence Data [Online]. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/. 2010.
35. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30(15):2114–20.
36. Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. Bioinformatics. 2013;29(8):1072–5.
37. Hunt M, Mather AE, Sánchez-Busó L, Page AJ, Parkhill J, Keane JA, et al. ARIBA: rapid antimicrobial resistance genotyping directly from sequencing reads. Microb Genom. 2017;3(10):e000131.
38. Gupta SK, Padmanabhan BR, Diene SM, Lopez-Rojas R, Kempf M, Landraud L, et al. ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. Antimicrob Agents Chemother. 2014;58(1):212–20.
39. Carattoli A, Zankari E, García-Fernández A, Larsen MV, Lund O, Villa L, et al. *In Silico* Detection and Tytypingf Plplasmidssing PlasmidFinder and Plplasmidumultilocusesequenceyping. Antimicrob Agents Chemother. 2014;58(7):3895–903.
40. Seemann T, Shovill. Faster SPAdes assembly of Illumina reads. https://github.com/tseemann/shovill. 2017.
41. Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics. 2014;30(14):2068–9.
42. Carver T, Harris SR, Berriman M, Parkhill J, McQuillan JA. Artemis: an integrated platform for visualization and analysis of high-throughput sequence-based experimental data. Bioinformatics. 2012;28(4):464–9.
43. Madeira F, Madhusoodanan N, Lee J, Eusebi A, Niewielska A, Tivey ARN, et al. The EMBL-EBI Job dispatcher sequence analysis tools framework in 2024. Nucleic Acids Res. 2024;52(W1):W521–5.
44. Seemann T. Snippy: rapid haploid variant calling and core SNP phylogeny. 2015.
45. Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, et al. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. Nucleic Acids Res. 2014;43(3):e15–e.
46. Page AJ, Taylor B, Delaney AJ, Soares J, Seemann T, Keane JA, et al. SNP-sites: rapid efficient extraction of SNPs from multi-FASTA alignments. Microb Genom. 2016;2(4):e000056.
47. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 2014;32(1):268–74.
48. Lee I, Ouk Kim Y, Park S-C, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol MicroBiol. 2016;66(2):1100–3.
49. Letunic I, Bork P. Interactive tree of life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics. 2007;23(1):127–8.
50. Chalita M, Kim YO, Park S, Oh HS, Cho JH, Moon J et al. EzBioCloud: a genome-driven database and platform for microbiome identification and discovery. Int J Syst Evol Microbiol. 2024;74(6).
51. WHO. Improving the prevention, diagnosis and clinical management of sepsis. 2017;https://apps.who.int/gb/ebwha/pdf_files/EB140/B140_12-en.pdf
52. Sękowska A, Gospodarek-Komkowska E. New species of Bacteria in human infections. Postępy Mikrobiologii - Advancements Microbiol. 2019;58(1):29–34.
53. Panditrao P M, Panditrao P M. Pantoea dispersa it the next emerging monster in our intensive care units? A case report and review of literature. Anesthesia: Essays Researches. 2018;12(4):963–6.
54. Mahapatra A, Dhal S, Jena PP, Mohapatra A, Dash D, Padhee A. Neonatal septicaemia due to a rare bacterium: Pantoea agglomerans (case series). Pediatr Infect Disease. 2014;6(3):102–4.
55. Hagiya H, Otsuka F. Pantoea dispersa bacteremia caused by central line-associated bloodstream infection. Brazilian J Infect Dis. 2014;18(6):696–7.
56. Dargère S, Cormier H, Verdon R. Contaminants in blood cultures: importance, implications, interpretation and prevention. Clin Microbiol Infect. 2018;24(9):964–9.

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