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Case Report

Macrococcus caseolyticus in early-onset neonatal sepsis, Kassala, Sudan

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Abstract

Introduction: Neonatal sepsis, a clinical syndrome characterized by systemic signs of infection in newborn infants (< 28 days old), are a significant cause of neonatal mortality and long-term morbidity globally, particularly in low- and middle- income countries.

Case presentation: We report the first case of neonatal sepsis caused by *Macrococcus caseolyticus* in a 48-hours old newborn who attended to the intensive care unit (ICU) of the Kassala Specialized Hospital for Pediatrics in Sudan, with signs of severe bacterial infection. *M. caseolyticus* is an opportunistic pathogen normally associated with veterinary and food-borne infections. Empirical antibiotic therapy was promptly initiated following blood sampling and culture, resulting in recovery within 4 days. *M. caseolyticus* was identified by mass spectrometry and confirmed by whole genome sequencing. The isolated strain, KaM20, was resistant to tetracycline, due to the presence of the *tet(L)* gene; and harbored several virulence-associated genes. Phylogenetic analysis including *M. caseolyticus* genomes from the GenBank suggested an animal origin for KaM20.

Conclusions: This case presents a rare instance of neonatal sepsis caused by *M. caseolyticus*; indicating potential zoonotic transmission of this pathogen, through maternal or environmental exposure to animals in the rural household. The findings emphasize the need for increased awareness of zoonotic infections in neonatal care, particularly in regions where exposure to animals is common; and underscore the importance of understanding the complex interplay between host factors, environmental exposures, and microbial pathogens, in the development of neonatal sepsis; reinforcing the need of a 'One Health' approach in addressing emerging infectious diseases.

Key words: neonatal sepsis; *Macrococcus caseolyticus*; Kassala.

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Introduction

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection in newborn infants who are less than 28 days old [1]; and represent a significant cause of neonatal mortality and long-term morbidity globally, particularly in low- and middle-income countries [2].

It is categorized into two types: sepsis in neonates before 72 hours of life (early-onset sepsis, EOS), and sepsis in neonates after 72 hours of life (late-onset sepsis, LOS) [1].

Typically, EOS indicates in utero exposure to infectious agents and transmission from maternal flora, whereas LOS refers to acquisition of infection after birth in healthcare settings or the community. The manifestation and severity of the condition are influenced by factors such as timing of exposure, infectious agent load, infant's immune status, and virulence of the specific pathogen [1]. The "gold standard" for diagnosis is a positive blood culture [2].

In addition to the classic pathogens involved in EOS and LOS [1], emerging pathogens are increasingly challenging the clinical management of neonatal sepsis [3–5]. In this case report we highlight the emergence of an atypical pathogen, *Macrococcus caseolyticus* (*M. caseolyticus*), associated with an EOS at the Neonatal Care Unit in Kassala Specialized Hospital for Children in Sudan.

M. caseolyticus is an opportunistic mammalian pathogen that is frequently detected on the skin of

various animals, as well as in milk/cheese or meat products, and is primarily associated with veterinary-related infections [6]. *M. caseolyticus* and its subspecies (*M. caseolyticus* subsp. *caseolyticus* and *M. caseolyticus* subsp. *hominis*), share genetic similarities with the genus *Staphylococcus*, but possess smaller genomes and lack the virulence genes present in *Staphylococcus aureus* [7], implying lower pathogenic potential.

Nevertheless, recent studies have shown that some *M. caseolyticus* strains, besides being responsible for infections in animals [8–10], have also been isolated from human clinical samples associated with infections including vaginitis, cervicitis, and vulvitis; and display antibiotic resistance [11]. In addition, *M. caseolyticus* strains harboring transferable genetic elements linked to methicillin resistance and other antibiotics [12–14], and thus acting as reservoirs for antibiotic-resistant genes (ARGs), have been identified. This poses a substantial threat to human health by potentially facilitating the transmission of ARGs to *Staphylococcus* species [15]. Our report focuses on concerns regarding the potential threat to human health posed by *M. caseolyticus*.

Case Report

A prospective hospital-based cross-sectional study was initiated in January 2023 at the Neonatal Intensive Care Unit (NICU) of Kassala Specialized Hospital for Pediatrics in Sudan, within the framework of Agenzia Italiana per la Cooperazione allo Sviluppo (AICS) project of the University of Sassari (AID 012162). The aim of this project was to assess the prevalence of neonatal septicemia, along with its bacterial profile, antibiotic susceptibility patterns, and the associated contributing factors. The project received ethical approval from the Health Research Ethics Committee of the Ministry of Health in Kassala State [Ref. 25/2022]. The study's progression is currently halted due to the ongoing civil war.

As a part of this study, we report a case of neonatal sepsis at the NICU that occurred in March 2023 where a 48-hours-old neonate was admitted displaying high-grade fever, jaundice, and vomiting. The baby was born at home in a suburban area of Kassala, in a household where animals typically lived in close proximity to humans. The baby was born through spontaneous vaginal delivery without complications. The baby exhibited normal crying and started breast feeding immediately after birth. However, after 48 hours, the baby developed high-grade fever, jaundice, vomiting, and signs of respiratory distress.

Results and discussion

The vital signs of the baby indicated a pulse rate of 110 bpm, respiratory rate of 66 cycles per minute, temperature of 38.3 °C, weight of 3.2 kg, and erythema around the umbilicus. The mother was asymptomatic; aged 33 years; and had 5 previous pregnancies, of which the most recent resulted in a fresh stillbirth with no apparent cause.

A blood sample was immediately collected by venipuncture, under strict aseptic conditions employing chlorhexidine 0.5% in 70% ethanol, and aseptically inoculated at 1:10 ratio directly into a 25 mL pediatric brain-heart infusion broth bottle (HiMedia, Modautal, Germany). Empirical antibiotic therapy with intravenous cefpodoxime was promptly initiated, leading to the baby's recovery in 4 days.

The inoculated blood culture bottle was maintained at a temperature below 25 °C and transported to the microbiology laboratory of Kassala University within 2 hours for bacterial isolation. Upon arrival, the bottle was promptly incubated aerobically with agitation at 35 °C overnight. Sub-culture was carried out within a safety cabinet, onto blood agar and chocolate agar plates (Liofichem, Teramo, Italy) and incubated both aerobically and in a CO_2 enriched atmosphere at 35 °C overnight. Bacterial growth was observed at 24 hours. Gram-positive bacteria were observed in pairs and clusters on Gram-stained slides, and showed a larger than normal *Staphylococci* diameter. The isolates that were catalase-positive and coagulase-negative were identified as *M. caseolyticus* by matrix assisted laser desorption/ionization time-of-flight mass spectrometry MALDI/TOF Biotyper, (Bruker Billerica, USA) with a score of 1.881.

The species was confirmed by analyzing the 16S ribosomal RNA gene after whole genome sequencing. Bacterial DNA was extracted using the Blood and Tissue Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions, quantified by Qbit (Thermofisher Scientific, Waltham, USA), and sent to BMR Genomics (BMR, Padova, Italy), for sequencing on an Illumina Miseq platform (Illumina, San Diego, USA) in paired-end format (2 x 300 bp). Adapter trimming and preprocessing were performed with the Fastp v0.23.2 preprocessor [16] in order to remove residual polymerase chain reaction (PCR) primer sequences, and filter low quality bases ($Q < 30$) and short reads (length < 150 bp). Profiling with MetaPhlan v4.0.1 [16] computational tool was employed in order to evaluate the sample content, including the identification of possible contaminants. Genome assembly was performed with the St. Petersburg

genome assembler (SPAdes) v3.15.5 [17] and assemb evaluation with the Quality Assessment Tool f.. Genome Assemblies (QUAST) v5.2.0 [18] and the Benchmarking Universal Single-Copy Orthologs (BUSCO) v5.4.3 software [19]. Gene prediction was carried out with the Prokka v1.14.6 software [20] and genes annotation with the tool eggNOG-mapper v2.1.7 [21]. The genome features of the *M. caseolyticus* strain, named KaM20 (accession number ERZ23867643), are summarized in Table 1, and the virulence and resistance genes prediction and annotations are summarized in Supplementary Tables 1 and 2, respectively.

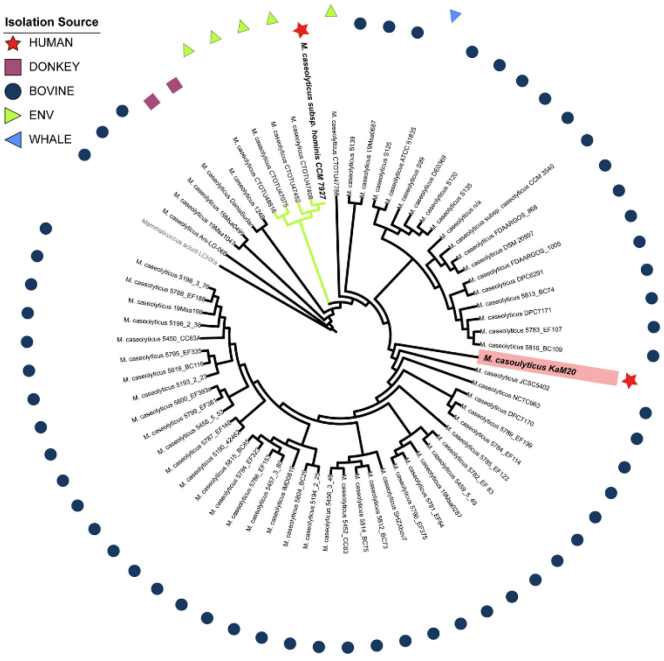
The 16S ribosomal RNA gene was analyzed using the National Center for Biotechnology Information (NCBI) BLAST tool [22] and showed 100% identity with various *M. caseolyticus* strains, including the *M. caseolyticus* subsp. *caseolyticus* CCM3540, and 99.86% identity with *M. caseolyticus* subsp. *hominis* subsp. nov. CCM7927. Finally, the KaM20 strain was conclusively classified as *M. caseolyticus* by calculating the average nucleotide identity (ANI) which was > 98% [23]. Antimicrobial susceptibility testing was performed using the Microscan4 system with the POS MIC STA 36 panel (Beckman Coulter, Brea, USA), following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) interpretive criteria for coagulase-negative *Staphylococci*. Antimicrobial resistance genes prediction was performed with the Antibiotic Resistance pipeline ABRicate v1.0.1 [24] against 5 antibiotic resistance databases: NCBI, CARD ARG ANNOT, Resfinder and MEGARES.

The *M. caseolyticus* KaM20 strain was phenotypically resistant to tetracycline (MIC > 2), associated with the presence of *tet(L)* gene (Supplementary Table 2) encoding for the tetracycline efflux protein TetL; and susceptible to all other tested antibiotics. Moreover, the aminoglycoside O-nucleotidyltransferases (ANT6) resistance determinant conferring resistance to streptomycin (not included by the European Committee on Antimicrobial Susceptibility Testing or the Clinical and Laboratory Standards Institute in *Staphylococcus* spp breakpoints) was detected (Supplementary Table 2). The *sdrM*, *norB*, and *sepA* genes which encode antibiotic efflux pumps and which can confer resistance to fluoroquinolone antibiotic, disinfecting agents, and antiseptics; were also noted (Supplementary Table 1). Antibiotic resistance genes like *tet(L)* limit treatment options, especially in resource-limited settings where tetracycline is still widely used for bacterial infections due to its affordability.

Moreover, despite the Pathogen Finder [25] analysis predicting *M. caseolyticus* strain KM20 as non-pathogenic for humans (score = 0.193), 19 virulence genes were detected by the Virulence Factor Database (VFDB) [26], 8 of which, including the capsule gene clusters (cap), were also present in the *M. caseolyticus* subsp. *hominis* CCM7927 strain isolated from a human vaginal smear [11] (Supplementary Table 1), but were absent in the non-pathogenic strain JCJS5402. Additionally, the detection of the biofilm-associated gene (*veg*), which can enhance adhesion to host tissues and protect against immune responses; together with the hemolysin III family gene (*hyl-III*), linked to increased virulence by promoting cell lysis and tissue damage, also described in strain CCM7927 [11]; and other pathogenic genes previously described in the pathogenic SDLY strain from commercial broiler chickens [27] (Supplementary Table 1); suggests the virulence potential of our *M. caseolyticus* isolate.

In order to determine the genetic relatedness and clarify the origin of the pathogen, a phylogenetic tree based on pangenome single nucleotide polymorphisms (SNPs) [28] was built by comparing the whole genome of *M. caseolyticus* KaM20 to the other 65 *M.*

Figure 1. Maximum likelihood phylogenetic tree based on pan-genome SNPs retrieved from 67 *Macroccoccus caseolyticus* genomes, including the *M. caseolyticus* KaM20 strain described here (highlighted).



The *Mammaliicoccus sciuri* strain LCHXa was used as outgroup. Isolation sources are indicated with shapes and colors as indicated in the figure legend. Green branches indicate the clade grouping the isolates of environmental origin and the *M. caseolyticus* subsp hominis CCM 7927.

caseolyticus species and subspecies strains isolated from human, animals, animal meat and milk, and environment; and one *Mammaliicoccus sciurii* strain LCHXa as an outgroup. All sequences are available in the NCBI database.

Phylogenetic analysis performed using the Interactive Tree of Life (iTOL) v6 [29] suggested an animal origin for the *M. caseolyticus* KaM20 strain, clustering with other *M. caseolyticus* strains originating from animal sources (bovine or chicken), including the *M. caseolyticus* subsp. *caseolyticus* strain CCM3540. *M. caseolyticus* KaM20 appeared not related to the *M. caseolyticus* isolated from the environment, including the *M. caseolyticus* CCM7927 strain which was the only other strain isolated from a human clinical specimen (Figure 1). These findings underscored significant genomic variability among *M. caseolyticus* strains.

As previously reported, *M. caseolyticus* exhibits genome plasticity, which is the ability of the *M. caseolyticus* genome to adapt and evolve through the acquisition of genetic material, in response to environmental conditions, such as antibiotic exposure or host interactions; leading to the emergence of more virulent or resistant strains [11]. In our case, the infection was likely zoonotic in origin; most possibly transmitted through maternal exposure during delivery, since the mother's vaginal colonization by *M. macrococcus* cannot be ruled out. The other possibility is environmental contamination from goats and/or geese living in the household. Previous reports of *M. caseolyticus* in goats' meat and milk [30,31], and its association with human vaginal infections [11] support the plausibility of this hypothesis.

Conclusions

The emergence of *M. caseolyticus* as a causative agent of neonatal sepsis poses a concerning public health challenge. This case underscores the importance of advanced diagnostic technologies in identifying atypical bacteria, and highlights the importance of thoroughly understanding the dynamics of zoonotic pathogens, particularly in regions where exposure to animal reservoirs is common.

The clustering pattern and genomic variability of *M. caseolyticus* have practical implications for understanding and managing its transmission. The association of the strain *M. caseolyticus* KaM20 with strains isolated from animals suggests a potential animal-to-human transmission route, emphasizing the need for stringent hygiene practices among those in contact with animals. Conversely, the clustering of the only other human strain with environmental isolates highlights the importance of controlling environmental reservoirs to prevent human infections. The genomic plasticity observed in *M. caseolyticus* enable it to acquire traits permitting the colonization of various hosts; thus, complicating the efforts to develop targeted interventions.

Finally, the findings reported here emphasize the connection among human, animal and environmental health; reinforcing the critical need for a 'One Health' approach to effectively tackle emerging infectious diseases, especially in rural settings. Enhanced surveillance and research to understand this pathogen's adaptability and to devise effective prevention and treatment strategies, tailored to its diverse transmission pathways and reservoirs, is needed.

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Conflict of interests

No conflict of interests is declared.

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Annex – Supplementary Items

Supplementary Table 1. Virulence and resistance genes (32/2328) obtained with Prokka v1.14 extrapolated from full genes prediction and annotation of KaM20 (Accession number ERZ23867643).

ID	Contig-ID	start	end	strand	length_bp	gene	product	orf
EPFJKLAN_00314	EPFJKLAN_1	275635	276471	+	837	*tlyA*	Hemolysin A	orf00305
EPFJKLAN_00364	EPFJKLAN_1	319777	320223	+	447	*ndk*	Nucleoside diphosphate kinase	orf00354
EPFJKLAN_00419	EPFJKLAN_1	369689	370360	+	672	*arlR_1*	Response regulator ArlR	orf00409
EPFJKLAN_00554	EPFJKLAN_2	2067	3206	-	1140	*epsN*	Putative pyridoxal phosphate-dependent aminotransferase EpsN	orf00967
EPFJKLAN_00556	EPFJKLAN_2	3824	4423	-	600	*epsL*	putative sugar transferase EpsL	orf00969
EPFJKLAN_00557	EPFJKLAN_2	4426	5688	-	1263	*wecC_1*	UDP-N-acetyl-D-mannosamine dehydrogenase	orf00970
EPFJKLAN_00560	EPFJKLAN_2	8106	8675	-	570	*dapH_2*	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-acetyltransferase	orf00973
EPFJKLAN_00563	EPFJKLAN_2	11156	12277	-	1122	*wbpI*	UDP-2,3-diacetamido-2,3-dideoxy-D-glucuronate 2-epimerase	orf00976
EPFJKLAN_00564	EPFJKLAN_2	12280	13389	-	1110	*wbjC*	UDP-2-acetamido-2,6-beta-L-arabino-hexul-4-ose reductase	orf00977
EPFJKLAN_00565	EPFJKLAN_2	13406	14431	-	1026	*capD*	UDP-glucose 4-epimerase	orf00978
EPFJKLAN_00566	EPFJKLAN_2	14457	16244	-	1788	*pglF*	UDP-N-acetyl-alpha-D-glucosamine C6 dehydratase	orf00979
EPFJKLAN_00567	EPFJKLAN_2	16262	16879	-	618	*ywqD*	Tyrosine-protein kinase YwqD	orf00980
EPFJKLAN_00579	EPFJKLAN_2	28649	29905	-	1257	*wecC_2*	UDP-N-acetyl-D-mannosamine dehydrogenase	orf00992
EPFJKLAN_00742	EPFJKLAN_2	194464	195303	-	840	*lgt*	Phosphatidylglycerol--prolipoprotein diacylglyceryl transferase	orf00734
EPFJKLAN_01110	EPFJKLAN_3	183793	184542	+	750	*uppS*	Ditrans,polycis-undecaprenyl-diphosphate synthase	orf00785
EPFJKLAN_01329	EPFJKLAN_5	9536	10525	+	990		Lipoate--protein ligase 1	orf01451
EPFJKLAN_01522	EPFJKLAN_6	34081	35223	+	1143	*mnaA*	UDP-N-acetylglucosamine 2-epimerase	orf01500
EPFJKLAN_01622	EPFJKLAN_7	31213	32235	-	1023	*malR*	HTH-type transcriptional regulator MalR	orf01608
EPFJKLAN_02126	EPFJKLAN_15	30926	31732	-	807	*yclP*	Petrobactin import ATP-binding protein YclP	orf02073
EPFJKLAN_01988	EPFJKLAN_12	24736	24999	-	264	*veg*	Protein Veg	
EPFJKLAN_02099	EPFJKLAN_15	7634	8302	+	669		hypothetical protein	
EPFJKLAN_00568	EPFJKLAN_2	16993	17670	-	678	*cap8A*	Capsular polysaccharide type 8 biosynthesis protein cap8A	
EPFJKLAN_00640	EPFJKLAN_2	94943	97522	-	2580	*clpB*	Chaperone protein ClpB	
EPFJKLAN_00801	EPFJKLAN_2	255310	256647	-	1338		hypothetical protein	
EPFJKLAN_00890	EPFJKLAN_2	344327	345802	-	1476		Fimbrial subunit type 1	
EPFJKLAN_01051	EPFJKLAN_3	123100	124782	-	1683	*rqcH*	Rqc2 RqcH	
EPFJKLAN_01514	EPFJKLAN_6	27713	28789	+	1077	*prfA*	Peptide chain release factor 1	
EPFJKLAN_01668	EPFJKLAN_7	77480	77950	+	471	*sepA_1*	Multidrug resistance efflux pump SepA	
EPFJKLAN_01763	EPFJKLAN_9	8041	9435	+	1395	*norB*	Quinolone resistance protein NorB	
EPFJKLAN_02298	EPFJKLAN_29	1768	3144	-	1377	*tet(L)*	tetracycline efflux MFS transporter Tet(L)	
EPFJKLAN_02296	EPFJKLAN_29	4	852	-	849	*aadK*	Aminoglycoside 6-adenylyltransferase	
EPFJKLAN_01672	EPFJKLAN_7	81495	82793	+	1299	*sdrM*	Multidrug efflux pump SdrM	
EPFJKLAN_01668	EPFJKLAN_7	77480	77950	+	471	*sepA_1*	Multidrug resistance efflux pump SepA	

Yellow background: IDs of virulence genes and orf identified by VFDB; Grey background: IDs of virulence genes detected manually; Red font: virulence genes also detected in *M. caseolyticus* sub *hominis* CCM7927 from vaginitis infection [11]; Red background: IDs of genes described in the pathogenic *M. caseolyticus SDLY* strain from broiler chickens [12]; Orange background: resistance genes.

Supplementary Table 2. Predicted antimicrobial resistance genes identified using ABRicate, including details such as scaffold identifiers, gene names, coverage and identity percentages, reference databases, accession numbers, and gene products.

SEQUENCE	Strand	End	Strand	Gene	Coverage	% Coverage	% Identity	Database	Accession	Resistance
NODE_29_length_3615_cov_61.612239	1768	3144	-	(Tet)tetL	1-1377/1377	100	99,93	argannot	FN435329:1-1377	
NODE_29_length_3615_cov_61.612239	1768	3144	-	TETL	1-1377/1377	100	99,93	megares	MEG_7095	
NODE_29_length_3615_cov_61.612239	1768	3144	-	tet(L)	1-1377/1377	100	99,71	card	M11036:0-1377	Tetracycline
NODE_29_length_3615_cov_61.612239	1768	3144	-	tet(L)	1-1377/1377	100	99,93	ncbi	NG_048203.1	Tetracycline
NODE_29_length_3615_cov_61.612239	1768	3144	-	tet(L)_2	1-1377/1377	100	99,93	resfinder	M29725	Doxycycline;Tet
NODE_29_length_3615_cov_61.612239	4	852	-	(AGly)str	1-849/849	100	99,53	argannot	X92946:18908-18060	
NODE_29_length_3615_cov_61.612239	4	852	-	ANT6	1-849/849	100	99,88	megares	MEG_1001	
NODE_29_length_3615_cov_61.612239	4	852	-	str	1-849/849	100	99,88	ncbi	NG_048080.1	Streptomycin
NODE_29_length_3615_cov_61.612239	4	852	-	str_2	1-849/849	100	99,88	resfinder	FN435330	Streptomycin