Isolation of *Janthinobacterium lividum* from early onset neonatal sepsis patients in Malaysia

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Abstract

Background: The term early onset neonatal septicaemia (EONS) refers to invasive bacterial infections that primarily involve the blood stream of neonates during the first 3 days of life. Although early onset neonatal septicaemia is relatively uncommon, it may be associated with case fatality rates of 15-30% and substantial morbidity in surviving infants.

Objectives: This study describes an unusual septicaemia cases with *Janthinobacterium lividum* in neonatal Intensive Care Units.

Methods: Bacterial causes of early onset neonatal sepsis in Kuala Lumpur Hospital Malaysia were investigated using broad range 16S rDNA PCR and sequencing. The bacterial DNA was isolated directly from blood without pre-incubation. All samples collected were equally cultured and incubated in automated BACTEC system.

Results: Two hundred and fifty two neonates were recruited in this study with mean (SD) gestational age of 35.9. Neonates with *J. lividum* infection lacked microbiological evidence of septicaemia as their blood culture yielded no bacterial growth. However, the PCR analysis of these samples yielded 1100bp corresponding to bacteria species.

Conclusion: This study demonstrates the value of PCR in detecting bacteria where special growth requirement is involved.

Keywords: *Janthinobacterium lividum*, neonatal septicaemia, neonatal intensive care units.

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Introduction

Neonatal sepsis is usually characterized by bacteraemia in addition to clinical symptoms resulting from infection with microorganism or its toxic products. Depending on the time of onset it can be classified as early onset (EONS) when sepsis occurs within the first 3 days of life or late onset (LOS) if symptom appears beyond 72 hours of life. The incidence of EONS is globally estimated at 3 million cases per live birth[1]. The incidence of EONS in South-East Asia is 4-10 per 1000 live birth[2]. The most frequently encountered organisms in the cases of EONS as reported from previous studies were Group B *Streptococcus* (GBS), *Escheria. Coli* and *Klebsiella* species[3,4].

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Early onset neonatal sepsis is often the most common disease presumably diagnosed in the neonatal intensive care units (NICU). This is mainly because of the signs and symptoms of disease are non-specific; the gold standard for diagnosis of neonatal sepsis is blood culture. However, the incubation period of 24-48 hours needed to report preliminary blood culture result, a low bacterial copy and inability to obtain sufficient amount of blood sample from neonates in addition to fastidiousness and

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special growth requirements exhibited by some bacteria and prior maternal antibiotic administration makes blood culture less suitable in the diagnosis of EONS. Molecular assays for detection of neonatal blood stream pathogens are promising and can serve as rapid and sensitive methods of diagnosing neonates with sepsis. Thus, it reduces the prolong hospital stay; lessen the overall medical cost and the emotional burdens of the families. A negative blood culture is commonly reported especially in cases of early onset neonatal sepsis in NICU, despite the clinical features suggestive of sepsis. Nonetheless, these neonates might not actually be free of infection, but the special growth requirement of the offending pathogen might have been the resulting cause of culture negative result. This study described an unusual septicaemia cases with *Janthinobacterium lividum* in neonatal intensive care units. The outcome of the study may lead to an in-depth analysis and modification of current approach of frequently reported negative blood culture result.

Material and methods

Ethical approval

This study was approved by the National Medical Research Register –National Institute of Health (NMRR-NIH) NMRR-ID: NMRR-12-631-10838 and the Research Ethic Committee of Universiti Putra Malaysia reference number: UPM/FPSK/100-9/2-MJKEtikaPen (Lect JPED_ April (12)01.

Patients

Study population included neonates of age 0 to 48 hours old who were admitted to the neonatal intensive care units (NICUs) between November 2012 and February 2014.

Inclusion criteria

Neonates who were clinically diagnosed as having sepsis and those who presented with at least one of the signs and symptoms of neonatal sepsis such as tachypnea (respiratory rate > 60 breath per minute), presence of flaring, grunting, chest recessions, fever (> 380 C), poor feeding, cyanosis, seizure, lethargy, etc. were included into the study.

Exclusion criteria

All babies born prior to 32 weeks of gestation and/or with birth weight of less than 1500gm were excluded. Neonates who were later found to have perinatal asphyxia, congenital cyanotic heart disease or multiple congenital abnormalities to avoid confounding factors as well as numerous missing hospital data were also excluded from the study.

Clinical data

Extensive dataset was collected using a standardized data extraction form. Additional data were extracted from the maternal hospital records in a retrospective study.

Specimen collection

At the end of all clinical evaluation by the consulting clinician on duty, two hundred and fifty two blood specimens (4ml) were drawn from eligible neonate prior to antibiotic administration. Approximately, one millilitre (1 ml) of the blood sample collected was inoculated into paediatric blood culture bottle and transported to microbiology laboratory for immediate incubation in automated BACTEC system. Positive samples when alerted were identified and processed accordingly by the laboratory staff in charge and results remained unknown to the researcher until the end of the study. From the remaining blood, 0.5ml was transferred into standard 0.5ml K3 EDTA sample tube and sent to the research laboratory at the Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences Universiti Putra Malaysia for molecular based identification.

The remaining 2ml of blood of each blood sample was transferred into standard 2ml vacutainer K3 EDTA tube and transported to the haematology laboratory Hospital Kuala Lumpur and were processed for automated full blood count (FBC) within one hour of sample receipt. Manual blood film for immature to total neutrophil ratio was obtained from a pair of slides prepared per sample. The preparation of slides was done in accordance with the Clinical and Laboratory Standard Institute (CLSI) protocol. White cell count (WBC) differential count was done on each film by a qualified haemato-pathologist and medical laboratory scientist. After staining, the monolayer at the tail end of the smear was examined using 100X (oil immersion objective). Manual absolute neutrophils count (ANC) was calculated as automated corrected WBC count (if required) X (% segmented neutrophils + % Immature granulocytes/100. Immature granulocytes consist of promyelocytes, myelocytes, and metamyelocytes and bands cells. Cells were considered as band if there was no nuclear segmentation.

The blood samples for molecular study were stored in the refrigerator at 4^0C for 72 hours or until there was a clear separation into plasma and red cells layers. The plasma was carefully removed into a sterile 1.5ml tube and stored at -20^0C, until further analysis. Deoxyribonucleic acid (DNA) was extracted from 200µl of the resulting plasma using QIAamp DNA mini kit (Qiagen Inc., Hilden, Germany) according to manufacturer's guidelines. The extracted DNA was eluted in 100µl elution buffer and stored at -20^0C until further analysis. DNA amplification was carried out in Biometra thermocycler (Biometra GmbH, Germany). Polymerase chain reaction (PCR) comprised of two oligonucleotide universal primers 5'-TGAAGAGTTTGATCATGGCTCAG-3' and 5'-TCGTTGCGGGACTTAACC-3 (IDT Technology, Singapore) as previously reported[5]. The PCR products amplified were electrophoresed in 1.2% gel at 70V for 90 minutes. The gel was visualized using gel documentation system (Bio Rad Laboratory Inc.) Products at the expected band size of 1100bp were considered positive. Bacterial DNA extracted from American Type Culture Collection (ATCC) *Staphylococcus aureus* was used as positive control. Negative control consisted of all PCR components except the template DNA.

The resulting products were purified using PCR purification kit (Vivantis® Technologies, Sdn Bhd, Malaysia) as per manufacturer's instruction.Purified products were sequenced commercially. Bidirectional sequencing was performed on the 1100bp 16S rDNA core region of PCR positives samples with forward primer 5'-TGAAGAGTTTGATCATGGCTCAG-3' and reverse primer 5'-TCGTTGCGGGACTTAACC-3(DNA Sequencer ABI 3730-XL, 1st BASE Company, Singapore). Traces were analysed with Applied Bioscience (ABI) sequence-analysis software, version 5.2.0. The good quality sequences were aligned using alignment tool in the SDSC Biology work bench 3.2. The aligned sequences were blasted against a quality controlled 16S rRNA gene library (Sepsis test-blast.net) to identify the organism. Genus and species identification were presumed to be correct for the isolate with sequence identity of ≥97% to reference strain in the data base.

Development of primers for species-specific PCR assays

We retrieved sequence of the for *J. Lividum* chitinase (Chi 69) gene complete cds (U07025) from the GenBank database, Designed primers specific for *J. lividum*. Primers JF'-5'-GACAAATCCGAATTCGCCCG-3' and JR'-5'-CGCCGCCTTCAACTTTTTCA-3'. These primers were optimised to amplify 151bp between positions 1305-1456 of the chitinase gene in *J. lividum*. Bacterial DNA from samples whose broad range PCR was positive for *J. lividum* were amplified with these specific primers. The PCR reactions of 25µl volume consisted of 12.5µl of master mix (Promega, USA), 0.2µM of each primers, 10µl of template DNA and 2µl of sterile milliQ water. The thermocycling condition included an initial denaturation at 94^0C for 4 minutes 35 cycles of 94^0C for 60 seconds, 51^0C for 30seconds, 72^0C for 2 minutes and a final extension at 72^0C for 10 minutes. The PCR products were ran in 1.2% gel and electrophoresed at 70V for 90 minutes. The gel was visualized using gel documentation system (Bio Rad Laboratory Inc.) Products at the expected band size of 151bp were considered positive. Amplification products were directly sequenced as mentioned earlier and blasted against the Basic Local Alignment Search Tool (BLAST) search algorithm.

Antibiotic resistance gene assays

The PCR primers for the antibiotic resistance blaZ and aac (6')-aph (2') that encodes for amino glycosides-modifying enzyme which confers resistance to Penicillin and Gentamycin were used to determine the susceptibility of *J. lividum* to antibiotic administered to the neonates under study. BlaZ-F'-5'-ACTTCAACACCTGCTGCTTTC-3' and blaZ-R'-5'-TGACCACTTTTATCAGCAACC-3' amplifies 173bp of blaZ. While aac(6')-aph(2')-F'-5'-TTGGGAAGATGAAGTTTTTAGA-3' and aac(6')-aph(2')-R'-5'-CCTTTACTCCAATAATTTGGCT-3' amplifies 174bp of aac(6')-aph(2') resistance gene. PCR amplifications were performed following standardized protocol described previously[6].

Data analysis

All data from the data extraction forms were entered into Statistical Package for the Social Sciences (SPSS) database. Data analysis was by descriptive and inferential statistics using SPSS for Windows, Version 20.0. Armonk, NY: IBM Corp.

Results

General data

There were 252 neonates who had their clinical data and blood sample collected for blood culture, PCR and other laboratory analysis. Among these, 22 had *J. lividum* in their blood samples. Details of the demographic characteristics of neonates with *J. lividum* infection are presented in Table 1.

Table 1: Demographic and birth characteristics data of neonates with J. lividum septicaemia (n = 22)

Sex	Mode of delivery	Gestational age(Weeks)	Birth weight (kg)	Age at presentation(Hours)	Reason for admission
Male	SVD	33.0	1.46	1.00	Prematurity and Presumed sepsis
Male	SVD	39.0	2.73	13.00	Presumed sepsis
Female	LSCS	35.0	3.42	28.00	Presumed sepsis
Male	Instrumental	38.0	3.30	1.00	others
Female	SVD	33.0	1.96	1.00	Presumed sepsis
Female	SVD	39.0	2.99	20.00	Presumed sepsis
Male	Instrumental	39.0	3.31	7.00	Presumed sepsis
Male	LSCS	34.0	1.45	1.50	Respiratory distress syndrome
Female	LSCS	39.0	2.90	44.00	Presumed sepsis
Female	Instrumental	39.0	3.27	1.00	Presumed sepsis
Female	LSCS	37.5	3.17	24.00	Presumed sepsis
Male	SVD	33.0	1.76	1.00	Prematurity and Presumed sepsis
Male	LSCS	38.0	2.95	1.00	others
Male	LSCS	35.0	1.89	24.00	Prematurity and Presumed sepsis
Male	LSCS	35.0	1.81	24.00	Prematurity and Presumed sepsis
Female	SVD	38.0	2.60	2.00	Presumed sepsis
Female	LSCS	34.0	1.52	2.00	Prematurity and Presumed sepsis
Male	LSCS	34.0	1.77	2.00	Prematurity and Presumed sepsis
Male	LSCS	35.0	2.55	17.00	Presumed sepsis
Male	LSCS	35.0	2.55	3.00	RDS and Presumed sepsis
Male	LSCS	38.0	4.35	1.50	Prematurity and Presumed sepsis
Female	LSCS	35.0	2.90	1.00	Prematurity and RDS

SVD: Spontaneous Vaginal Delivery, LSCS: Lower segment Caesarean section, RDS: Respiratory distress syndrome

The gestational age of neonates with *J. lividum* septicaemia ranges from 32-40 weeks and the mean ± SD gestational age is 35.9weeks ± 2.56. Among these, 54.5% were born premature with tachypnea as the commonest presenting clinical characteristics as presented in Table 2.

Thirteen (59.1%) Males and nine (40.9%) Females were positive for *J. lividum* infection. About half of these newborns required oxygen support with seven (32%) had non-invasive ventilator support. All neonates were discharged alive and without any neonatal complication. Risk factors predisposed to neonatal sepsis is presented in Table 3. It was determined that leaking liquor, urinary tract infection and intrapartum fever are among the risk factors associated with *J. lividum* neonatal sepsis.

Table 2: Clinical presentation of neonates with J. lividumsepticaemia (n = 22)

Clinical presentation	n (%)
Tachypnea	16 (72.7)
Grunting	7 (31.8)
Chest recession	7 (31.8)
Fever (>38°C)	3 (13.6)
Nasal flaring	6 (27.3)
Vomiting	4 (18.8)
Lethargy	2 (9.1)
Prematurity	12 (54.5)
Low birth weight	3 (13.6)

*Some neonates presented more than one clinical sign; Prematurity: Baby born less than 37 week

Table 3: Risk factors associated with *J. lividum* early onset neonatal septicaemia (n-22)

Risk factors	n (%)
Leaking liquor >18hurs	6 (27.3)
Premature prelabour rupture of membrane	2 (9.1)
Meconium stained liquor	4 (18.2)
Foul smelly liquor	1 (4.5)
Chorioamnionitis	1 (4.5)
Maternal urinary tract infection	6 (27.3)
Intrapartum fever	4 (18.2)

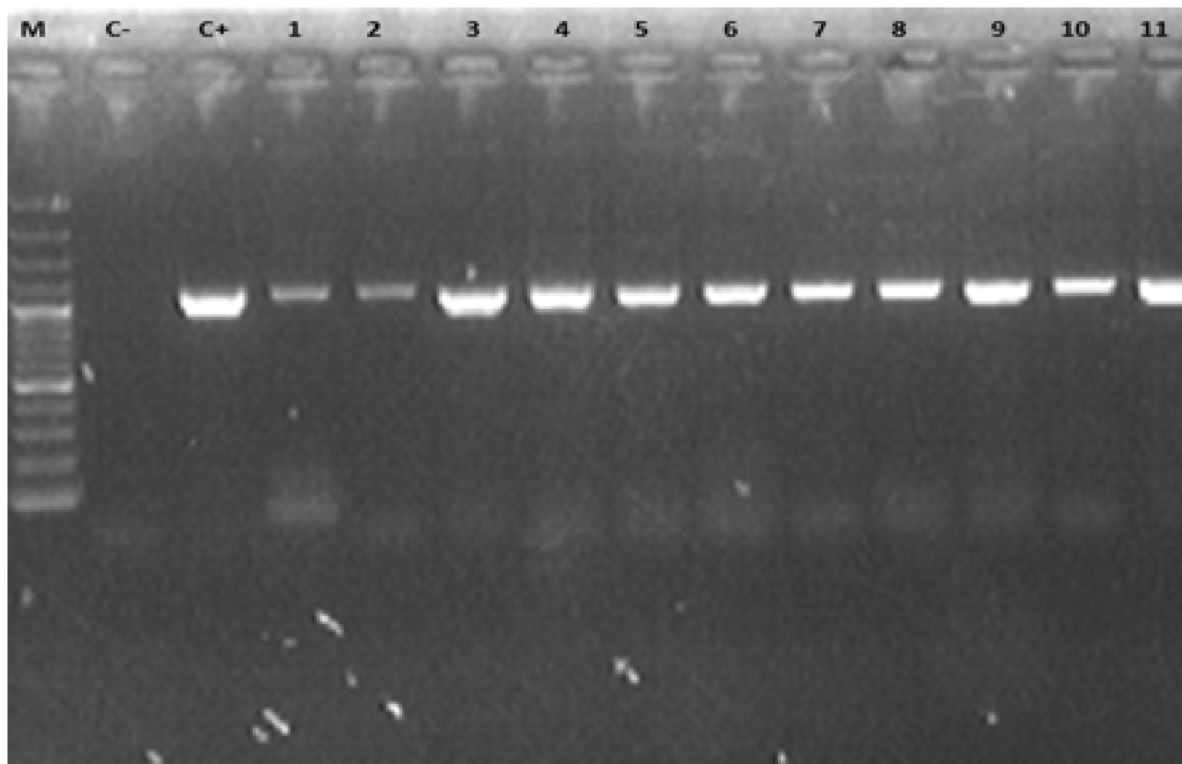

Figure 1: Amplification of 1100bp 16S rRNA from blood samples (M: 100bp marker)

PCR and sequencing

All 22 neonates with *J. lividum* infection lacked microbiological evidence of septicaemia as their blood culture yielded no bacterial growth. However, the PCR analysis of these samples yielded 1100bp corresponding to bacteria specie or genera as shown in Figure 2. Following 16S rDNA sequencing and BLAST search algorithm of GenBank a quality controlled 16S rRNA gene library (Sepsis test-blast.net), the best matches and sequence homologies were that of *J. lividum* (Figure 3).Other samples negative by PCR were equally negative by culture.

Table 4 shows the analysis of laboratory parameters of the 22 neonates with *J. lividum* septicaemia, analysis of the commonly utilised hematologic parameters in diagnosing neonatal septicaemia showed abnormality in some haematological parameters and C-reactive protein (CRP) with majority of neonates having parameters within normal limit.

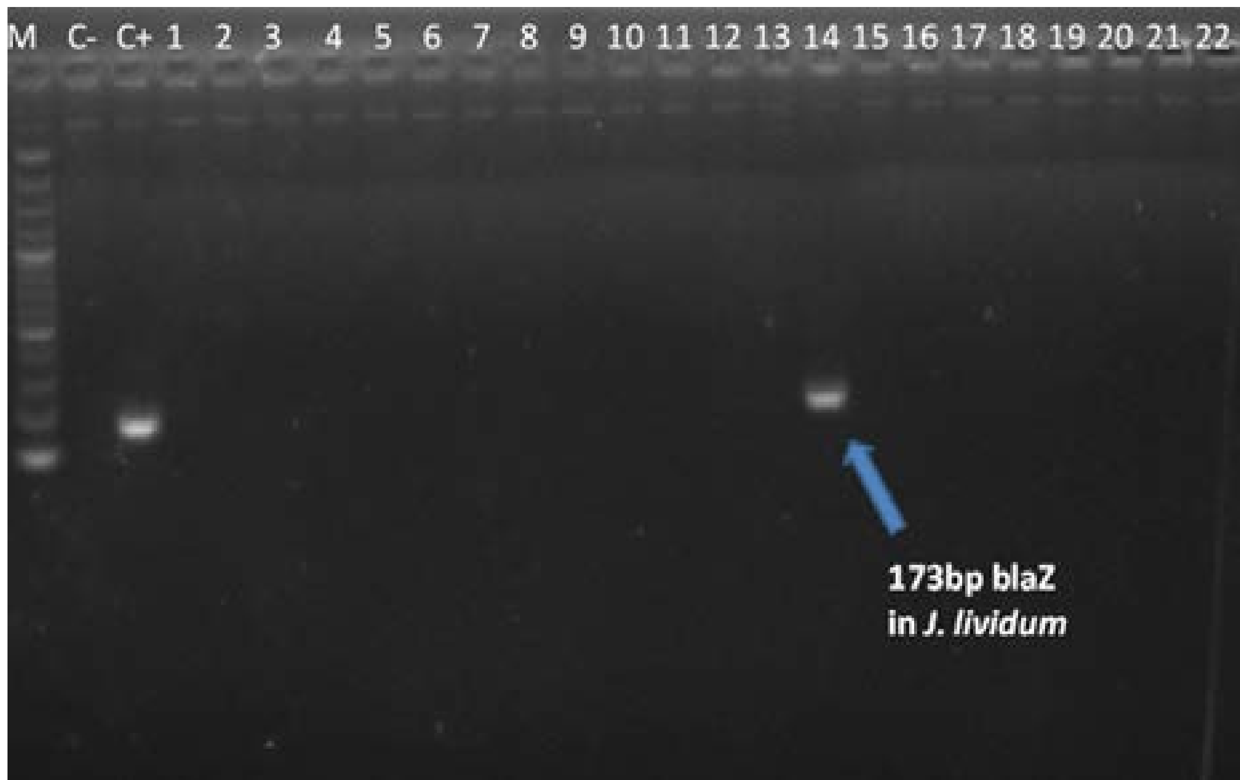

Figure 2: Amplification of 173bp blaZ resistance gene in *J. lividum* (M: 100bp marker)

Figure 3: Amplification of aac-(6')-aph-(2') resistance gene in *J. lividum* (M: 100bp marker)

Table 4: Individual laboratory parameters of neonates with J. lividum septicaemia (n = 22)

WBS (cells/L)	ANC (cells/L)	nRBC (%)	Platelets (cells/L)	IT ratio IG/TNC	CRP (mg/dl)
2810.00	1690.00	1.00	22000.00	.21	1.70
21350.00	15120.00	1.00	217000.00	.09	.20
24300.00	1230.00	6.00	282000.00	.17	.40
22470.00	15270.00	1.00	278000.00	.09	1.60
21780.00	16420.00	.00	167000.00	.28	6.00
6500.00	3200.00	15.00	90000.00	.09	.80
19460.00	10900.00	.00	282000.00	.02	20.80
25550.00	19930.00	5.00	249000.00	.10	.30
24190.00	16940.00	.00	327000.00	.11	2.80
6260.00	2620.00	.00	195000.00	.05	.20
26040.00	19040.00	1.00	170000.00	.03	.20
11760.00	5710.00	3.00	159000.00	.02	1.60
11760.00	5720.00	5.00	162000.00	.13	1.60
16140.00	12720.00	4.00	232000.00	.29	2.90
10020.00	30030.00	9.00	196000.00	.06	.20
9710.00	3310.00	15.00	145000.00	.05	.20
11540.00	4710.00	6.00	145000.00	.13	7.80
15130.00	9180.00	.00	56000.00	.02	1.90
19900.00	10020.00	11.00	298000.00	.08	1.90
8850.00	820.00	7.00	224000.00	.13	.20
8180.00	820.00	.00	224000.00	.04	.20
2810.00	1690.00	1.00	22000.00	.21	1.70

Reference values: White blood cell count (WBC): or ≤5000/mm^3 or ≥ 25000, 30000 and 21000/mm^3 (at birth, 12-24 hours and 48hours).Absolute Neutrophils Count (ANC): Corrected WBC count (if correction required) × (% Segmented neutrophil + immature granulocytes)/100 Nucleated red blood cells (nRBCs): Immature to total neutrophil ratio: > 0.2 Platelets: ≤ 150,000C-reactive protein (CRP): > 5.0mg/dl

Antibiotic resistance assay

All extracted DNA of *J. lividum* strains were tested for resistance to Penicillin and Gentamicin by means of the blaZ and aac (6')-aph (2') specific PCR assays in Figure 4. Gentamycin-resistant was not found in all strains analyzed. However, one strain showed the 173bp of blaZ (gene in penicillin resistant) PCR amplicon (Figure 3). Subsequent DNA homology searches performed using the DNA sequences confirmed the result.

```
Janthinobacterium lividum strain HC-3 16S ribosomal RNA gene, partial sequence
Sequence ID: gb|KF993615.1| Length: 1433 Number of Matches: 1

Range 1: 7 to 1050 GenBank Graphics                     ▼ Next Match ▲ Previous Match
Score            Expect  Identities        Gaps         Strand
1912 bits(1035)  0.0     1042/1045(99%)    1/1045(0%)   Plus/Plus

Query  7    GGGGGCTGCTTACCATGCAGTCGAACGGCAGCACGGAGCTTGCTCTGGTGGCGAGTGGCG  66
            |||||||||||| |||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7    GGGGGCTGCTTAACATGCAGTCGAACGGCAGCACGGAGCTTGCTCTGGTGGCGAGTGGCG  66

Query  67   AACGGGTGAGTAATATATCGGAACGTACCCTAGAGTGGGGGATAACGTAGCGAAAGTTAC  126
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  67   AACGGGTGAGTAATATATCGGAACGTACCCTAGAGTGGGGGATAACGTAGCGAAAGTTAC  126

Query  127  GCTAATACCGCATACGATCTAAGGATGAAAGTGGGGGATCGCAAGACCTCATGCTCGTGG  186
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  127  GCTAATACCGCATACGATCTAAGGATGAAAGTGGGGGATCGCAAGACCTCATGCTCGTGG  186

Query  187  AGCGGCCGATATCTGATTAGCTAGTTGGTAGGGTAAAAGCCTACCAAGGCATCGATCAGT  246
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  187  AGCGGCCGATATCTGATTAGCTAGTTGGTAGGGTAAAAGCCTACCAAGGCATCGATCAGT  246

Query  247  AGCTGGTCTGAGAGGACGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGG  306
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  247  AGCTGGTCTGAGAGGACGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGG  306

Query  307  GAGGCAGCAGTGGGGAATTTTGGACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGA  366
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  307  GAGGCAGCAGTGGGGAATTTTGGACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGA  366

Query  367  GTGAAGAAGGCCTTCGGGTTGTAAAGCTCTTTTGTCAGGGAAGAAACGGTGAGAGCTAAT  426
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  367  GTGAAGAAGGCCTTCGGGTTGTAAAGCTCTTTTGTCAGGGAAGAAACGGTGAGAGCTAAT  426

Query  427  ATCTCTTGCTAATGACGGTACCTGAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCG  486
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  427  ATCTCTTGCTAATGACGGTACCTGAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCG  486
```

Figure 4: The sequence of the purified PCR product showing 99% identity to the Gene Bank Sequence (Accession No: KF 993615.1) as *Janthinobacterium lividum* Sequence the homology of 16S ribosomal RNA fragment with HC-3. (Length = 1433)

Table 5: Laboratory parameters of neonates with J. lividum septicaemia (n = 22)

Laboratory parameter	results
WBC(cells/L) median [range]	13360 [23330]
ANC (cells/L), median [range]	9180 [29210]
nRBC (%), median [range]	2[15]
Platelets (cells/L) mean ± SD	195652 [78963]
I:T ratio (%), median [range]	0.882 [0.27]
CRP(mg/dl), median [range]	1.6 [20.60]

WBC = white blood cells; ANC = absolute neutrophil count; nRBC = nucleated red blood cells; I: T = proportion of immature –to-total neutrophils

Discussion

J. lividum is one of the common bacteria found in the environment. Closely related bacteria *Chromobacterium violaceum* has been reported in several human infections, no available report on *Janthinobacterium lividum* contributing to EONS. Despite the fact that this result has not been previously described, the researchers are convinced about its acceptability because all the sequences of the amplicon were 100% identical to *J. lividum* sequence as shown in Figure 4. Basic description of genus *Janthinobacterium* was given by Sneath in 1984. It is a Gram negative, motile aerobic bacterium found in soil and waters (rivers, lakes and springs)[7-9] and it was also reported in the spoilage of pasteurized milk[10]. Unlike other bacteria, *J. lividum* is unique in its incubation temperature. Although 96% of the Janthinobacterium strain can grow at 4^0C, the optimal temperature for growth in the laboratory is 25^0C. Therefore, it is not surprising if cultures for these bacteria yielded no growth since *Janthinobacterium* can never grow at 37^0C[11]. Molecular studies such as PCR and sequencing on new-born had demonstrated more microbial causes of septicaemia than that shown by conventional culture method[12-14]. *J. lividum* formally known as *Chromobacterium lividum* is a violet-pigment producing gram negative rod which is motile and survived in oxygen rich environment. It is mostly found in the tropical and subtropical regions usually in soil, water, and occasionally in putrid food as well as on the leaf nodule of a plant.

There was a report on fatal human infection with these bacteria from Thailand causing hospital acquired septicaemia in adult[15]. It was reported to be among unreported bacteria causing ventilator associated pneumonia on patients who were intubated and on mechanical ventilation[16]. This observation was supported by previous report of *J. lividum* among the ignored bacterial flora inhabiting medical devices and work places within the NICU[17]. Interestingly, this study was conducted in neonates aged less than 48 hours old who presented with sign and symptoms of infection prior to their admission. Bacterial causes of EONS unless otherwise proven, are acquired from the mother rather than the environment. It might have been assumed that the infection is acquired within the NICU from medical devices, since most of the neonates under study were either on Nasal Continuous Positive Airway Pressure (NCPAP) or ventilator support, however, all neonates were admitted to the neonatal intensive care units immediately after birth and were already having symptoms suggesting early onset sepsis.

The infection could have been acquired maternally as there were reports of these bacteria from the cases of bacterial vaginosis[18]. Earlier than this, *J. lividum* was reported as part of vaginal microbial communities within individuals[19]. Most of the study population who were found to have positive PCR analysis for *J. lividum*, had at least one of the risk factors that predisposed them to infection including leaking liquor, meconium stained liquor, maternal urinary tract infection and premature rupture of membrane. These factors may cause them to be vulnerable to infection since it is confirmed to be inhabitant of women's genital tract.

Analysis of blood culture negative clinical samples using broad range real time PCR and subsequent sequencing, detected *J. lividum* in cases where bacterial infection were highly suspected but remained negative by culture[20].The bacteria were not isolated by culture in this study. This was similarly reported by other researchers[16-19]. The possible reason why *J. lividum* failed to be isolated by conventional culture method may be attributed to the incubation temperature. All incubations are usually done at 37^0C while *J. lividum* only grow at 25^0C[21]. Several earlier studies reported the identification of *J. lividum* by 16S rRNA PCR except study by Patijanasoontorn et al[15] who isolated the bacteria by culture. However did not reveal the culture technique. A study from Thailand reported septicaemia caused by *J. lividum* with high fatality rate. Nonetheless, no patients with *J. lividum* infection in this study died as a result of sepsis. The potential reason could be due to prompt antibiotic administration to all clinically suspected sepsis among neonates who were admitted to NICU. To further confirm this, DNA samples from 22 *J. lividum* positive samples were subjected to antibiotic resistance assays. However, none of the 22 samples was found to have resistance gene to Gentamicin while one strain was found to have resistance gene to Penicillin but not having resistance gene to Gentamycin. These neonates were treated with the two antibiotics based on the suspicion of having neonatal sepsis.

Limitations

Tracing the source of these bacteria would have revealed useful information about its role in causing early onset neonatal sepsis, but this was not evaluated mainly due to financial constraint.

Conclusion

J. lividum has not been reported in neonates or any NICU in Malaysia. This is the first report of this specie in neonatal blood. It is mostly of nosocomial origin and possibly colonized mothers in the hospital. Although there were no cases of neonatal infection with these bacteria, nosocomial infection with *J. Lividum* clearly occurred in a hospital intensive care unit in Thailand[15]. Therefore, this highlights the need for infection control practices by the health care workers to prevent future occurrence. Furthermore, these findings demonstrate the value of PCR in clinical settings. Though PCR cannot displace culture, it certainly offers improvement in the diagnosis of bacterial infection especially when bacteria with special growth requirement are involved.

Disclosure statement

None to declare.

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