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RESEARCH PAPER

Diagnosis of Bacterial Meningitis in Cerebrospinal Fluid Negative Cultures using of Universal 16S rRNA Gene in Erbil City, Iraq.

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A B S T R A C T:
Culture-dependent techniques can occasionally fail to detect bacterial infections in body fluid samples, especially when antimicrobial drugs have been administered previously. Amplification of nucleic acid specially 16S rRNA using PCR (polymerase chain reaction), has been utilized for identifying besides distinguishing of numerous sorts of bacterial infections in patients. The present work investigated the employ of 16S rRNA gene and DNA sequencing to recognize as well as detect the pathogens in statuses with negative cultures of cerebrospinal fluid (CSF). In CSF culture, eight (7.2%) samples were discovered to be positive; including Staphylococcus sciuri (2 isolates), Staphylococcus xylosus (one isolate), Enterococcus casseliflavus (2 isolates), Escherichia coli (2 isolates), Micrococcus luteus (one isolate). For universal PCR assay, 37 (33.6%) of the samples had bacterial 16S rRNA gene positivity (directed CSF 16S rRNA PCR). DNA sequencing performed for 17 out of 37 samples, which identified Bacillus cereus (5 cases), Pseudomonas sp. (4 cases), Sphingomonas sp. (2 cases), S. sciuri, Acinetobacter spp. and Brevundimonas sp. (one of each), and uncultured bacteria (3 cases). The results designate that 16S rRNA gene PCR/sequencing has seemed to be a useful tool for diagnosis of culture-negative CSF samples. PCR/sequencing appears to be a supportive diagnostic tool for the culture-negative CSF samples. In accordance with the findings, the culture was less effective than PCR in diagnosing of bacterial meningitis. In bacterial meningitis patients, high CSF protein (>50mg/dl), low CSF glucose level (>40mg/dl), high CSF White blood cell (WBC) count (>100cell/mm3) and high polymorphonuclear leukocyte (PMNL) (>50%) were diagnosed.

KEY WORDS: Bacterial meningitis, Cerebrospinal fluid, culture negative and 16S rRNA gene.
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1. INTRODUCTION:

Meningitis is a significant healthiness issue which affects humans worldwide, especially children. The prevalence of infection rates differs by nation, province, age stage, as well as infectious agent. In underdeveloped nations, the occurrence of meningitis in babies below the five years range from 0.07 percent to 2.5 percent. It is a fatal condition (Zhao et al., 2021).

In Iraq, the annual incidence rate (IR) of laboratory-confirmed bacterial meningitis was 1.47 per 100 000 population (Al-Sanouri et al., 2021). Meningitis may be initiated by different factors, the most common of which are bacterial, such as, *Neisseria meningitidis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Listeria monocytogenes*.

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Group B Streptococcus, *Staphylococcus aureus*, and *Escherichia coli*, or viruses, fungi, parasites, as well as bodily injury, malignancy and medicines (Mani et al., 2007, Afifi et al., 2007). Microorganisms can enter the meninges through bacteremia, congenital meningeal deficiency, cranial damage, the nasopharynx, or the ears (WHO, 2017).

Bacterial meningitis (BM) is a severe condition that injures and inflames the meninges, spinal cord, and brain parenchyma which dramatically increases mortality and morbidity (Abdelkader et al., 2017). Pathogen early detection in clinical specimen is an important stage for better detection as well as treatment of bacterial contagions (Grif et al., 2012, Ramya et al., 2018). Clinical samples have typically been processed by traditional microbiology using

phenotypic identification techniques and cultures of microorganisms on different media (Rampini et al., 2011, Culbreath et al., 2019).

It is essential for precise diagnosis of invasive infections, such as meningitis, empyema, pericarditis and peritonitis, to isolate bacteria by culture from sterile body fluid (NSBF) (Altun et al., 2015). There are numerous methods for diagnosing bacterial meningitis, but CSF culture is indeed regarded the "gold standard" (Saravolatz et al., 2003, Wu et al., 2013). Nevertheless, culture frequently defeat to identify clinically significant pathogens because of stringent growth conditions, in some circumstances culture techniques are insensitive, especially when the patient has been pretreated with antibiotics; furthermore, there is the drawback of the turn-around time before results are available (Ahmadi et al., 2015, Grif et al., 2012, Wu et al., 2013) As a result, quick and precise molecular techniques have been proposed to close these gaps, especially in challenging cultural negative contexts (Saravolatz et al., 2003, Tuyama et al., 2008, Abdeldaim et al., 2010, Sacchi et al., 2011)

In latest years, the PCR assay has recently been utilized in clinical test center to recognize the microorganism from normally aseptic places, specifically in situations of fastidious bacteria increasing slowly that have been pretreated with antibiotics and bacteria that are biochemically difficult to identify (Yoo et al., 2020). PCR-based analyses of CSF have been proposed as an immediate, sensitized and private investigative inspection for BM. Such technique operates autonomously of microbial progression, and the discovery of tiny quantities of DNA from nonviable bacteria might possibly aid in diagnosis in cases where cultures are negative (Schiess et al., 2021).

The objective of the current work was to indicate of bacterial meningitis in Erbil city, Iraq, using the 16S rRNA gene, PCR and sequencing in cases when bacterial infection is supposed while the culture remain negative.

2. MATERIALS AND METHODS

2.1. Samples Collection:

The present study was directed in Raparin Pediatric Teaching Hospital (Erbil City, Iraq) over one year from June 2021 to June 2022. CSF samples were collected by senior doctors who were in charge of patients with suspected circumstances of meningitis. The specimens of CSF were submitted to the laboratory of Microbiology. The specimens were promptly inoculated onto macConkey, blood, and chocolate agar plates at least 2 hours after collection. The Petri dishes were incubated in an aerobic and CO2 supplemented environment at 35–37°C for 24–48 h. All isolated bacteria were recognized depending on their cultural characteristics (Sharma et al., 2022), then subsequently verified using VITEK-2 Compact System (bioMérieux, France).

An overall of 110 CSF specimens were used in the study (1 day -15 years), admitted to Raparin Pediatric Hospital, displaying clinical symptoms (fever, vomiting, headache, photophobia, and irritability were the clinical requirements for patient inclusion, along with neck rigidity, seizures, the Brudzinski sign, the Kernig sign, besides focal neurological marks), unusual CSF cellular and chemical findings that are indicative of bacterial meningitis (Fouad et al., 2014).

The following were among the analytic laboratory necessities for bacterial meningitis: White blood cell count greater than 100 cells per mm3, percentage of neutrophil greater than 50%, level of protein greater than 50 mg/dL and glucose concentration less than 40 mg/dL (Heydari et al., 2016). Patients whose cultures came back negative had their data registered, and their frozen CSF samples (-20C^{o}) were extra analyzed by conventional PCR assay.

2.2 Molecular Diagnosis

Molecular analysis was achieved on 49 out of 110 CSF samples (depending on clinical manifestation and positivity of CSF parameters) using particular gauges, which were specimens displaying no growth of bacteria on culture media but elevated leucocyte count or a preponderance of PMNLs, as well as chemical analysis results (high protein level and low glucose concentration). By using the AddPrep Genomic DNA extraction kit, Korea, DNA was extracted. Traditional PCR was utilized to amplify the 16S rRNA gene with forward primer 536f 5′-CAGCAGCCGCGGTAATAC3′ plus reverse primer RP2 5′- ACGGCACCTTGTTACGACTT-3′ (Bioneer, Korea) (Bousbia et al., 2010).

Preparation the specimens for PCR: CSF specimens (50 µL - 1.5 mL) were centrifuged for

20 min at 12 000 g, (Welinder-Olsson et al., 2007), then from the pellet, DNA was extracted by a bacterial DNA preparation kit (AddPrep Genomic DNA extraction kit, Korea) according to the constructer's guidelines. DNA purity and concentration were measured by means of a NanoDrop Spectrophotometer. (ND-1000; NanoDrop Technologies, UK) at 260 nm and 260/280 nm, respectively.

The amplification was carried out in final volume of 50- µL comprising 5 µL extracted DNA. Amplification begun with an preliminary incubation for 15 min at 95°C to DNA denaturation then polymerase enzymes activation, followed by 35 cycles of heating for 1 min at 95°C, annealing for 30s at 62°C, and extension for 90s at 72°C. Amplification ended with an extension step for 10 min at 72°C (Bousbia et al., 2010). To determine the size of the PCR product, it was electrophoresed on a 1% agarose gel.

The refinement of the DNA amplicon was done by means of a Wizard® SV Gel and PCR Clean-Up System Kit (Quick Protocol, Promega, USA) according to manufacturer's instructions, to eliminate nonincorporated dNTPs, polymerase enzymes, boundless primers, salts, and other unpurities that were portion of the PCR reaction producing the DNA sequencing target. Finally, bacteria were known by comparing the acquired nucleic acid sequences to known sequences in the GenBank database by means of BLAST software, which is accessible on the National Center for Biotechnology Information website (BLAST) https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_SPEC=GeoBlast&PAGE_TYPE=BlastSearch.

2.3 Statistical Methods

Statistical analysis, Graph Pad Prism v 8.0.1 software was utilized. For categorical variables, two-sided Chi-square test analysis were done to examine the differences between the parameters with bacterial positive culture, Positive by PCR and negative cases.

3. RESULTS AND DISCUSSION

3.1. Results

Out of 110 CSF samples, 8 (7.2) cases were positive by culture, 37 (33.6) cases were positive using PCR and 45 (40.9%) were positive using both methods, besides the other cases 65 (59%) were negative by both methods as shown in Figure (1).

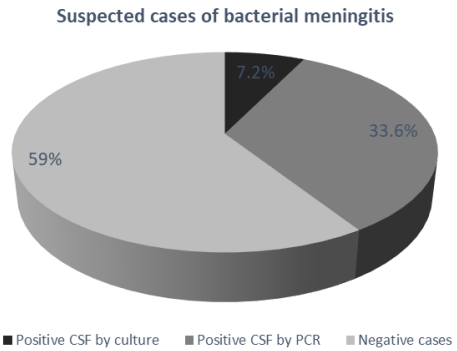

Figure 1: Percentage of bacterial meningitis cases confirmed by culture and PCR technique.

In CSF culture, eight (7.2%) samples were detected to be positive; the bacteria which isolated were *S. sciuri* (2 isolates), *S. xylosus* (one isolate), *E. casseliflavus* (2 isolates), *E. coli* (2 isolates) and *M. luteus* (one isolate). 37 (33.6%) of the samples tested positive for the bacterial *16S rRNA* gene using the universal PCR assay. (directed CSF *16S rRNA* PCR). DNA sequencing conducted on 17 out of 37 samples, which identified *Bacillus cereus* (5 cases), *Pseudomonas* sp. (4 cases), *Sphingomonas* sp. (2 cases), *S. sciuri*, *Acinetobacter* spp. and *Brevundimonas* sp. (one of each), and uncultured bacteria (3 cases), as shown in Table (1) and Figure (2).

Table (1) Direct 16S ribosomal RNA sequencing versus culture for diagnosing bacteria in cerebrospinal fluid specimens.

Pathogen identified	Clinical Identification	
	Culture & VITEK 2 System	16S rRNA /Sequencing
Staphylococcus sciuri	2	1
Staphylococcus xylosus	1	0
Micrococcus luteus	1	0
Enterococcus casseliflavus	2	0
Bacillus subtilis	0	5
Pseudomonas sp.	0	4
Acinetobacter spp.	0	1
Sphignomonas sp.	0	2
Brevundimonas sp.	0	1
Escherichia coli	2	0
Unidentified	0	3
Total	8	17

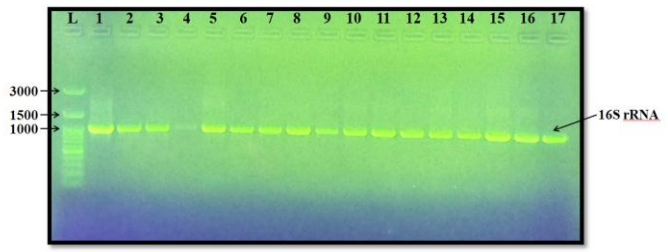

Figure 2: Universal PCR fragments for various kinds of meningeal pathogen (~1000) on 1% agarose gel. Lane L: Ladder (100bp); Lane 1-5: Bacillus cereus; Lane 6-9: Pseudomonas sp.; Lane 10-11: Sphingomonas sp.; Lane 12: Staphylococcus sciuri; Lane 13: Acinetobacter sp.; Lane 14: Brevundimonas sp. and Lane 15-17: uncultured bacteria.

The complete obtained sequences of the nucleotide of *16S rRNA* gene for *B. cereus*, *Pseudomonas* sp., *Acinetobacter* spp. and *S. sciuri* and presented in Figure (3). The sequences obtained were further analyzed using the blast search and alignment available at http://blast.ncbi.nlm.nih.gov/Blast.

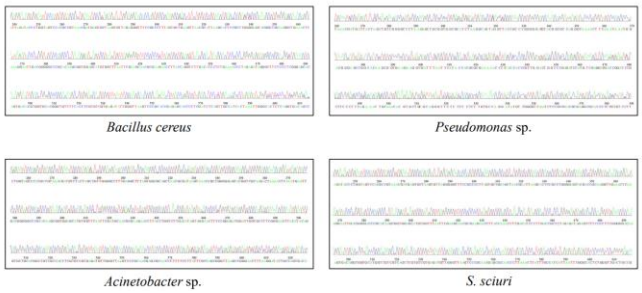

Figure 3: Peak of sequencing of *B. cereus*, *Pseudomonas* sp., *Acinetobacter* spp. and *S. sciuri*.

Table (2) and Figure (4) display the patients' demographic traits. Among the total patients, 48 (43.6%) were aged < one year and 62 (56.3%) were aged > one year (1-15 years). The distribution of sex was 57 (51.8%) male and 53 (48.1%) female. The most clinical manifestation of patients with suspected meningitis in this investigation were fever, headache, nausea, vomiting, diarrhea, cough, head rigidity, lethargy, seizure, dyspnea, cyanosis and abnormal movement. Also it was found that the increased level of WBC count, PMNL, high level of protein and low concentration of glucose in CSF samples were intensely related with bacterial meningitis in this study as shown in Table (3). A high relation was observed between bacterial meningitis cases and cytological parameters, as well as high relation indicated between bacterial meningitis cases and chemical parameters. From a statistical point of view, there was a significant difference between positive cultures with cytological parameters and chemical parameters (P<0.0001), as revealed in Figure (5, 6) respectively.

Table (2) Demographic characteristics of patients

Variables	Level	Suspected cases (110)	Confirmed positive cases (45)		Negative cases (65)
			By culture (8)	By PCR (37)	
Age	Less than 1 year	48	5	14	29
	More than 1 year	62	3	23	36
Gender	Male	57	6	21	30
	Female	53	2	16	35

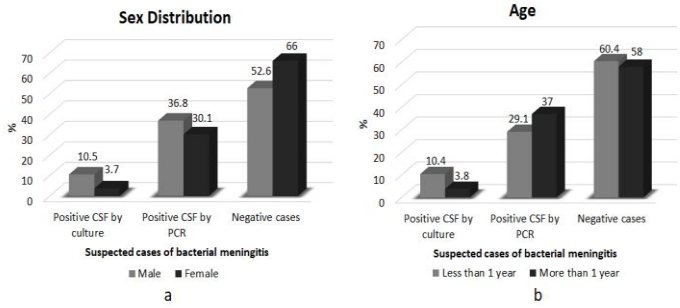

Figure 4: Percentages of (a) sex distribution and (b) age among positive and negative cases of bacterial meningitis.

Table (3) Laboratory findings in 110 neonates with and without bacterial meningitis.

CSF parameters	CSF Positive by culture	CSF Positive by PCR	CSF Negative culture
WBC			
- No cell	0	8	18
- < 10 (cells/mm^3)	0	13	28
- 10-100 (cells/mm^3)	0	8	17
- 100-1000 (cells/mm^3)	5	6	1
- > 1000 (cells/mm^3)	3	1	1
PMNL > 50%	8	7	2
< 50%	0	22	45
Protein > 50mg/dl	8	14	6
< 50mg/dl	0	22	59
Glucose < 40mg/dl	7	25	2
> 40mg/dl	1	11	63

CSF: Cerebrospinal fluid; PCR: Polymerase chain reaction; WBC: White blood cell; PMNL: Polymorphonuclear leukocyte.

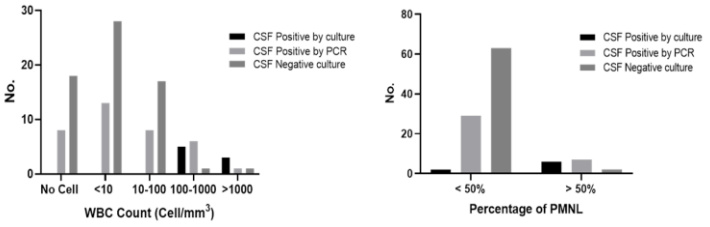

Figure 5: The relation between cytological parameters and positive bacterial culture in CSF (P<0.0001).

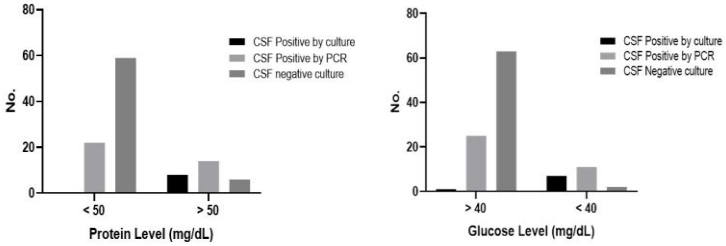

Figure 6: The relation between chemical parameters and positive bacterial culture in CSF (P<0.0001).

3.1. Discussion

Precise detection of the microorganisms which are answerable for acute infectious illnesses continues to be a major encounter for physicians, particularly when traditional microbiological examination is negative. For the perfect diagnosis and management of infections in body fluids, adequate, fast, and dependable lab testing (CSF) are necessary. In the current study, 65 of the 110 CSF samples taken were negative in culture around twelve months from 2021 to 2022, accounting for 59% of the overall number of specimens submitted.

In the present investigation, 110 CSF culture were positive in 7.2% (n = 8) patients, while the non-culture techniques (PCR) detected bacteria in 33.6% (n = 37) patients, Table (1) and Figure (1). The findings of earlier research are consistent with this low level of positivity in CSF culture (Amin et al., 2016, Nour and Alaidarous, 2018, Albuquerque et al., 2019, Zhao et al., 2021, Sharma et al., 2022). The reasons for a poor diagnostic value on CSF culture, are a low bacterial load (Başpınar et al., 2017) and the use of antibacterial drugs before collecting CSF (Ceyhan et al., 2014, Wu et al., 2013, Zhao et al., 2021), in addition to inadequate culture facilities such as a lack of special media for pathogenic bacteria, unsatisfactory fluid storage, autolytic enzymes being available, samples being chilled before plating, an improper or delayed inoculation, and an inadequate supply of transport media, and insufficiency in CSF specimen processing. Similarly to Nhantumbo and colleagues, this study discovered that a number of CSF samples (59%) were negative for the bacteria under investigation (Nhantumbo et al., 2015).

In present study, isolated bacteria were *S. sciuri* (2 isolates), *S. xylosus* (one isolate) *E. casseliflavus* (2 isolates), *E. coli* (2 isolates), *M. luteus* (one isolate) as in Table (1). In a study performed by Wang and Zhu (Wang and Zhu, 2021), they were indicated that E. coli was identified in 28 cases among the positive CSF cultures, and coagulase-negative staphylococci (CoNS) was discovered in 1 case, and stated that E. coli was the highest dominant pathogen as bacterial meningitis. In a research achieved by Devi and coworker's (Devi et al., 2017), they were investigated that only *E. coli* was detected among gram negative bacteria and Enterococcus spp. as well as CoNS were most commonly detected among the Gram-positive bacteria.

In a study, a previously healthy baby (twelve months old) who developed meningitis due to *M. luteus* (Gupta et al., 2019). In the other study, *E. cassiliflavus* was not found in CSF culture, and only one case of the bacterium was identified as bacterial meningitis in a 77-year-old female (Iaria et al., 2005).

Staphylococcus xylosus, a commensal bacteria found in the mouth cavities of animals, can cause serious infections. *S. xylosus* was noticed in the CSF of a 9 year-old boy who had been bitten by a dog on his right forearm and thigh and was bleeding from the wound (Singh and Jain, 2016). In each of the two studies, only one instance of *S. sciuri* was examined (Marsou et al., 1999, Chang et al., 2018).

Neisseria meningitidis, Haemophilus influenzae, Streptococcus pneumoniae and *Listeria monocytogenes* are the most often reported bacteria causing meningitis. However, they are not indicated in this study which agree with other studies (Gray and Fedorko, 1992, Zhao et al., 2021). It is unclear if *N. meningitidis*, *H. influenzae*, *S. pneumoniae*, and *L. monocytogenes* are exact causative agent of meningitis due to the absence of established methods for isolating them, or the patients may have been treated with antibiotics prior to lumber puncher (LP), and other reasons are that those bacteria are belonging to fastidious bacteria in which they are sensitive to chemical and physical factors in vitro, as well as the CSF specimens might not endure lengthy transit times or temperature changes while being

transported to a microbiology lab. (Gray and Fedorko, 1992, Zhao et al., 2021), these are all agree with current findings.

The current study merely conducted the PCR analyses on the samples that were culture-negative in order to conserve resources because the PCR outcomes wouldn't influence the consequence in circumstances where the results of positive culture were gained. All direct CSF PCR-positive samples in this study were discovered to be correlated either clinically or with CSF meningitis parameters, which is consistent with other study (Devi et al., 2017).

The PCR assay does not necessitate the existence of enormous amounts of organisms, provide rapid diagnosis, use a bit quantity of sample, as well as it could identify unviable, growing slowly, or fastidious organisms (Zhang et al., 1995, Welinder-Olsson et al., 2007). Bacterial meningitis agents are presently recognized by various PCR techniques with high specificity and sensitivity. However, these tests are not normally used for identification in hospitals in developing countries, with the exception of referral centers where expensive equipment is required (Wang et al., 2014). The PCR assay detected more children with bacterial meningitis than culture (37 cases versus 8 cases). As a result, PCR is useful for diagnosing bacterial meningitis, especially when patients are taking antibiotics. PCR results may be positive despite antibiotic pretreatment. PCR has the advantage of being quick; results are reported the same day, as opposed to the longer time required for culture. For cases of bacterial meningitis, PCR provides an early diagnosis, whereas for cases with negative results, PCR provides additional confirmation that antibiotics can be discontinued.

In addition to *16S rRNA* gene targeted sequencing, Yoo and colleagues (Yoo et al., 2020) found that diagnostic yield increased by 5.12%. A study achieved by Grif and colleagues (Grif et al., 2012), they were compared the traditional culture and the usage of universal 16S gene PCR/sequencing on various fluids in body. Their findings revealed that the PCR positivity ratio (34.6%) was greater than the bacterial culture positivity ratio (25%). They proposed two explanations for their findings: either fastidious microbes were more common in the specimens or the patients had recently received antibacterial treatment.

Next Generation Sequencing-identified species revealed a diverse bacterial population that included *Pseudomonas* sp. (10.5%), *Acinetobacter* sp. (4.9%) and *Bacillus* sp. (3.3%) which conducted by Men and colleagues (Men et al., 2020). Those bacteria which were identified by direct CSF *16S rRNA* PCR/sequencing were *Staphylococcus* spp. (1), *Staphylococcus sciuri* (1), *Bacillus cereus* (1), *Pseudomonas* sp. (1), GBS (1), *Acinetobacter baumannii* (3) and uncultured bacteria (5) which were performed by Devi and coworkers, similar to the present study (Devi et al., 2017).

In current study, 2 isolates of *Sphingomonas* sp. were detected by PCR/sequencing in CSF directly, while in other investigations it was detected by culture method as bacterial meningitis (Hajiroussou et al., 1979, Tai and Velayuthan, 2014, Bolen et al., 2015, Mehmood et al., 2018). Besides, *Brevundimonas* sp. was detected in one patient by direct CSF PCR/sequencing, while it was previously discovered in other studies by culture (Mondello et al., 2006, Karadag et al., 2012). *Sphingomonas* sp. together with *Brevundimonas* sp. were not previously thought to be important pathogens, but the most recent findings indicated that this notion has to be reconsidered.

Out of 110 cases in present study, 48 (43.6%) were under one year old, and 62 (56.3%) were over one year old (1-15 years), 53 (48.1%) females and 57 (51.8%) males made up the gender split. A study included 106 cases, of which 65 (61.3%) were male and 70.8% (75/106) were under 1 year old (Zhao et al., 2021). The ratio of male patients (20.8%) and the percentage of female patients (15.1%) differed significantly. Neonates (aged 1 month-1 year) and adults (aged 61-99 years) with poor immunity were most vulnerable to BM (Ali et al., 2021).

A study performed by Ali and colleagues (Ali et al., 2021), they were found that high percentage of elderly patients and young children with E. coli associated bacterial meningitis. Jiang and colleagues (Jiang et al., 2017) also discovered E. coli (28.5%) related circumstances in Chinese children's CSF. In another study found that E. coli (51.3%) is frequently the cause of bacterial meningitis in young babies (Nhantumbo et al., 2015).

Premature birth, being underweight, and also being infected with pathogenic microorganisms are the three most recognized

hazard aspects for meningitis in children, especially among newborns and infants (Sáez-Llorens and McCracken Jr, 2003, Osrin et al., 2004).

Children under one year are more likely to develop bacterial meningitis, because maternal antibodies cannot traverse the placenta after 32 weeks of pregnancy, or because the immune systems are weakened by the phagocytic activity of neutrophils and monocytes. Additionally, meningitis and sepsis develop at an early age in very low birth weight neonates as a result of bacterial infections caused by E. coli (Davis, 2018).

According to a study by Fish (Fish, 2008), there are more male infants with BM infections than female patients, and this disparity is caused by the fact that female newborns have stronger immune systems than male infants. Furthermore, estrogen raises the amount of Th1, resulting in a higher immune response against bacterial infections, whereas testosterone decreases T-cell production of the cytokines interleukin 4 and interferon (Fish, 2008, Araneo et al., 1991).

The most common clinical features of suspected cases of meningitis in this study were fever, headache, nausea, vomiting, diarrhea, cough, head rigidity, lethargy, seizure, dyspnea, cyanosis and abnormal movement, which agree with other studies (Garges et al., 2006, Peros et al., 2020, Zainel et al., 2021, Sameh et al., 2021, Jiang et al., 2017, Devi et al., 2017, Meiring et al., 2022, Nour and Alaidarous, 2018, Wang and Zhu, 2021). These CSF parameters laterally with clinical features and culture of bacteria rise the specificity, sensitivity and accuracy of the investigation by lots of folds in identifying of bacterial meningitis (Nigrovic et al., 2007).

The current study indicates that, individuals with bacterial meningitis had higher CSF cell, protein, and glucose levels. Inflammatory alterations in CSF, including the protein levels, sugar concentrations, WBCs, neutrophils, and lymphocytes, have been seen in various studies of BM patients; similar findings have been described in the current investigation (Ali et al., 2021, Babenko et al., 2021). Others have described normal CSF values in the presence of meningitis (Garges et al., 2006). Patients with bacterial meningitis exhibited considerably greater CSF TLC levels, PMN, as well as protein than patients with aseptic meningitis, but considerably lower glucose levels of CSF and lymphocytes than patients which have aseptic meningitis, which is consistent with the current study. Several investigations found comparable results (Makoo et al., 2010, Abdelkader et al., 2014). Similarly to earlier research, current analysis revealed that the group of CSF culture-positive had more leucocytes in their CSF specimen than the group of CSF culture-negative (Wang and Zhu, 2021, Sharma et al., 2022).

According to this finding, the majority of studies found lower CSF glucose concentrations and higher CSF protein in patients with BM (Martinot et al., 2018, Julián-Jiménez and Morales-Casado, 2019, Al-Dulaimy et al., 2020, Telano and Baker, 2021, Devi et al., 2017, Wang and Zhu, 2021).

According to Sáez-Llorens and McCracken, the main reasons for the elevated CSF protein level are the immune system response and the disturbance as well as raised bacterial blood-brain barrier permeability (Sáez-Llorens and McCracken Jr, 2003). The glucose disturbance dispersion and raised glucose utilization in reaction to bacterial infection caused the hypoglycorrhachia (CSF glucose <45 mg/dl) in bacterial meningitis (Chow and Troy, 2014).

Neonates with a positive CSF culture also had CSF protein levels that were noticeably high, which indicates a continuing inflammatory response. Increased interstitial vascular permeability during the acute stage of NBM enables pathogens to get across the blood-brain barrier. Teichoic acid and other inflammatory mediators are released by these infections, and these mediators trigger an immunological response. On CSF analysis, higher levels of protein were seen in the CSF as a result of inflammatory mediators such PMNL, cytokines and interleukins. Reactive oxygen species, however, are also produced as a result of the same immunological response. These very unstable free radicals might result in the production of damaging proteins, lipids, and nucleic acids. The central nervous system suffers damage when lipid levels are high and cerebral antioxidant levels are low (Barichello et al., 2013, Kim, 2010). As a result, the inflammatory cascade in NBM is both the reason for neurologic morbidity and attempts to keep the central nervous system (Wang and Zhu, 2021).

4. CONCLUSIONS

The ability to identify uncultivable or nonviable bacteria as well as fastidious pathogens as a result of prior antimicrobial therapy makes

16S rRNA gene PCR and sequencing a potent tool for detection of pathogens. PCR could be thought of as a complement to cultural characteristics. Molecular diagnosis is useful for diagnosing bacterial meningitis in suspected meningitis with negative cultures. Present meningitis like symptoms in negative cultures and PCR results indicate present agents rather than bacteria.

The accession number of all isolated by PCR assay are unfinished, that are at the Submission Type step in the Genbank.

Statements and Declarations

Conflicts of interest

The authors have not declared any conflict of interests.

Ethics approval

This research was approved by Human Research Ethics Committee of Science College/Salahaddin University/Ministry of Higher Education and Scientific Research, Erbil/Iraq.

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