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Traditional and molecular methods for diagnosing bacterial meningitis in Erbil city, Iraq

Niga Othman Hamaameen* , ***Adel Kamal Khider***

Department of Biology, College of Education, University of Salahaddin, Erbil, Iraq.
*Corresponding Author.

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Abstract

Bacterial meningitis is a leading cause of illness and death worldwide. It is crucial for clinical and public health care, as well as disease control, to identify the meningitis-causing agent promptly. Between June 2021-February 2022, a total of 100 cerebrospinal fluid (CSF) and blood samples were collected from suspected cases of meningitis admitted to Raparin Paediatric Teaching Hospital, Erbil city-Iraq. Cytochemical, cultural, and biochemical tests were conducted, and confirmed by molecular techniques. Bacterial culture findings were positive in 7% of CSF samples and just one positive among blood samples. The most common pathogens found by cultural characteristics and VITEK 2 Compact System were *Staphylococcus sciuri* in two cases 2%, *Staphylococcus xylosus* in one case 1%, *Escherichia coli* in two instances 2%, *Enterococcus casseliflavus* and *Micrococcus luteus* each in one case 1%. *Staphylococcus sciuri*, *Staphylococcus xylosus*, *Enterococcus casseliflavus* and *Micrococcus luteus* were first recorded as bacterial meningitis in Erbil/Iraq. All isolates were confirmed by PCR assay. All clinical isolates were screened for some antimicrobial sensitivity, meropenem and tobramycin have been shown to be totally resistant 100% to all isolated bacteria, furthermore, isolated *E coli* showed highly resistant 100 to cefotaxime, gentamycin, pencillin, ceftriaxone, rifampin, amoxicillin/clavulanic acid, ceftazidime, erythromycin, ampicillin, and clindamycin, while they were sensitive (100%) to amikacin and imipenem as well as all the gram positive bacteria were resistant 100% to optochin and sensitive (100%) to gentamycin, and trimethoprim. In bacterial meningitis patients, high C-reactive protein (CRP) >6 mg/dl, high CSF protein >50 mg/dl, low CSF glucose level <40 mg/dl and high leukocyte count >100 cells/mm^3 were all substantially diagnostic.

Keywords: Bacterial Meningitis, Blood Specimen, Cerebrospinal Fluid, Cultural characteristics, PCR.

Introduction

Meningitis is an infection of the subarachnoid space and leptomeninges and is commonly related to meningeal inflammation, which is induced by a number of microbial pathogens that is still a major cause of mortality and morbidity[1]. Meningitis can arise from a variety of reasons, the most common of which are bacterial, such as, *Haemophilus influenzae*, *Neisseria meningitides*, *Streptococcus*

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pneumonia, Group B *Streptococcus*, *Escherichia coli*, or viruses, as well as bodily injury, malignancy, as well as medicines[2-4].

Bacterial meningitis is a potentially fatal inflammation of the central nervous system that requires rapid medical intervention and is a significant reason for morbidity and death in children. In the affluent world, 5 percent fatality rates have been recorded, though in the underdeveloped world, the amount might be more than thirty percent. Even with proper care, morbidity and mortality can be significant. Clinicians must be able to detect the clinical manifestations of meningitis, as well as comprehend its therapy and prevention. The elimination of the invading organism from fluid surrounding the brain and spinal cord is fully reliant on antimicrobial treatment that must be started as momentarily as conceivable following the diagnosis of bacterial meningitis [4-9].

Immediate bacterial detection and treatment may reduce the number of fatalities and neurological sequelae. Antimicrobials are critical in lowering the mortality rate of infectious illnesses. Despite improvements in the healthcare system and medicines, the growing death rate is concerning [6].

Bacterial meningitis mediated by these microorganisms often manifests as fever, headache, neck stiffness, vomiting and photophobia in adults, children, and teenagers (aged 10-19 years). In neonates from 1 to 28 (days), babies (greater than one year), and small kids from 1 to 10 (years), the symptoms and indications are non-specific, including poor feeding lethargy, irritability, vomiting, and linked with fever [9-11].

For bacterial pathogen detection, the majority of medical laboratories use manual, automated, or semiautomated morphological approaches and commercial systems. Gram stain findings, colony characteristics, growth supplies, and biochemical and/or physiological functions are established, nevertheless, these features are not fixed and may alter as a consequence of tension or progression [12,13].

It is critical to distinguish between bacterial and nonbacterial meningitis while selecting therapy. Bacterial meningitis is a potentially fatal neurological illness that requires immediate parenteral antibiotics, as opposed to viral and aseptic meningitis, that have a better prognosis [14]. Polymorphonuclear leukocytosis, reduced level of glucose, and high levels of total protein are the most common CSF malformations in bacterial meningitis. The main characteristics of CSF malformations in viral meningitis include lymphocytic pleocytosis, usual glucose concentrations, and usual or faintly increased protein levels. Additional clinical tests are required to differentiate between bacterial and viral meningitis. Patients with bacterial meningitis frequently have an increased peripheral WBC count and CRP [11,15,16].

The aim of this article is to investigate the clinical profile of patients with bacterial meningitis in children aged 1 day to 15 years old, as well as to identify the most prevalent organism found in this group based on cultural characteristics, VITEK-2 Compact System and molecular method (PCR) through detection of some genes of isolated bacteria for further confirmation, and identify the antimicrobial susceptibility patterns of the isolated bacteria by using disc diffusion method. Performation of some biomarker tests such as C-reactive protein (CRP), protein level, sugar level and WBC count for meningitis patients.

Materials and Methods

Sample collection and bacterial isolation

This study was approved by the Human Research Ethics Committee of Science College/Salahaddin University/Ministry of Higher Education and Scientific Research, Erbil/Iraq. This study was authorized from June 2021 to February 2022, among pediatric patients ranging from one day to 15 years of age admitted to Raparin Paediatric Teaching Hospital, Erbil city/Iraq.

Blood specimens and CSF samples were taken. CSF samples were collected by senior doctors who were in charge of patients with meningitis suspicions.

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CSF samples were promptly inoculated onto macConkey, blood, and chocolate agar plates at the latest 2 hours after collection. The Petri dishes were incubated in an aerobic and CO_2 supplemented environment at 35–37°C for 24–48 h. Gram staining was achieved. All isolated bacteria were recognized depending on their cultural characteristics [7,17-19], then subsequently verified using VITEK-2 Compact System (bioMérieux, France).

Blood specimens were directly cultivated into blood culture bottles and transferred to the microbiology laboratory for overnight incubation at 35-37°C and subsequent culture on macConkey, blood and chocolate agar plates aerobically and in a CO_2 supplemented environment at 35–37°C for 24–48 hours. Microorganisms were further identified by VITEK-2 Compact System (bioMérieux, France).

Molecular confirmation of isolated bacteria

For additional validation, all positive CSF bacterial cultures were molecularly typed using the PCR method, which was chosen by detection of *uspA* gene for *E. coli*, *16S rRNA* gene for Staphylococci species and universal *16S rRNA* for *Micrococcus luteus* and *Enterococcus casseliflavus*. *E. coli* ATCC 25922 was used as a positive control. The primers used are shown in Table 1.

After inoculating 10 ml LB broth with bacterial culture for 24 hours at 37ºC in a shaker incubator, the pellet was microcentrifuged at 14000-16000 rpm to gain the pellet, then DNA from the pellet was extracted using a (AddPrep Genomic DNA extraction kit, Korea) according to the manufacturer's instructions. A NanoDrop ND-1000 Spectrophotometer was used to detect DNA quantitatively (Thermo Fisher Scientific, Labtech, UK).

The PCR reaction tubes were placed in a thermo-cycler machine, and DNA to *uspA*, *16S rRNA*, and Universal *16S rRNA* genes were replicated using the PCR program that has been designed in the thermo-cycler based on the temperature profile provided from each primer, as given in Table 2. In the electrophoresis unit, agarose gel electrophoresis was employed to identify the PCR product; 3µl of each product of PCR was electrophoresed on a 1 percent agarose gel to demonstrate PCR amplification.

Table 1. The primers used for amplification of all isolated bacterial meningitis.

Target gene	Nucleotide sequence (5`-3`)	Amplicon size (bps)	References
uspA	**F** CCGATACGCTGCCAATCAGT **R** ACGCAGACCGTAGGCCAGAT	884	[20]
16S rRNA	**F** GTTGACTGCCGGTGACAAAC **R** GCTGTTACGACTTCACCCCA	372	[21]
Universal *16S rRNA*	**F** GTTGACTGCCGGTGACAAAC **R** ACGGCACCTTGTTACGACTT	~ 1000	[22]

Table 2. The PCR programme used to amplify *uspA*, *16S rRNA* and Universal *16S rRNA* genes, respectively.

Target genes	PCR program							References
	Initial denaturation (ºC/min)	Denaturation (ºC/sec)	Annealing (ºC/sec)	Extension (ºC/sec)	Final extension (ºC/min)	Final store (ºC/min)	Cycles	
uspA	94/5	95/15	56/45	72/60	72/10	5/10	40	[20]
16S rRNA	94/5	95/30	59/45	72/40	72/5	5/10	35	[21]
Universal *16S rRNA*	95/15	95/60	62/30	72/90	72/10	5/10	35	[22]

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Antimicrobial Sensitivity Test

According to the standard technique suggested by the CLSI in 2020, the antimicrobial sensitivity test was achieved. Twenty three antimicrobials were used against both gram negative and gram positive bacteria such as amoxicillin/clavulanic acid (AMC)(30µg), azithromycin (AZM)(15µg), bacitracin (B)(20µg), ceftazidime (CAZ)(30µg), clindamycin (DA)(2µg), cefotaxime (CTX)(30µg), erythromycin (E)(10µg), trimethoprim/sulfamethoxazole (SXT)(25µg), imipenem (IPM)(10µg), meropenem (MEM)(10µg), ampicillin (AM)(25µg), rifampin (RA)(5µg), tobramycin (TOB)(10µg), vancomycin (VA)(30µg), amikacin (AK)(5µg), ceftriaxone (CRO)(30µg), penicillin (P)(10µg), ofloxacin (OFX)(30µg), ciprofloxacin (CIP)(5µg), nitrofurantoin (N)(300µg) trimethoprime (TMP)(25µg), optochin (OP) (5µg) and gentamycin (CN)(30µg). The transparency of the inoculums was set to 0.5 McFarland turbidity. Isolated bacteria were evenly distributed on Mueller-Hinton Agar plates, while fastidious bacterial cultures were cultivated on mueller-hinton agar plates enriched with 5% sheep blood. The antibiotic discs were evenly spaced on the bacterial infected plates. Ultimately, the plates were then incubated at 37°C for 24 hrs, and the results were measured by (mm) [23].

Cytological Examination, Chemical Analysis and Serological test

All samples were examined for leukocyte count by microscopic examination [24]. The samples were chemically analyzed (Cobas c 311) to identify the amounts of glucose and total protein. Measurement of C-reactive protein (CRP latex test kit) for quantitative estimation [25].

Statistical analysis:

Graph Pad Prism version 8.0.1 software was used for the statistical analysis. While, for categorical variables, two-sided Chi-square test analysis were done to examine the differences between the parameters and bacterial positive culture.

Results and Discussion

In this study, an overall of one hundred patients with suspected cases of meningitis was studied. 51 patients (51%) were males and 49 patients (49%) were females. Patients aged < 1 year were 55 (55%) and 45 patients (45%) were >1 year. Demographic characteristics of patients are shown in Table 3. All samples were collected during nine months from June, 2021 to February, 2022 in Raparin Paediatric Teaching Hospital, Erbil/Iraq. Gram staining was performed of CSF samples, in which 2 samples were gram negative bacteria and 5 samples were gram positive bacteria. The bacteria was confirmed in 7 (7%) cases by morphological cultural methods and VITEK-2 Compact System, while 93 (93%) of clinically suspected meningitis were negative by culture.

Table 3. Demographic characteristics of patients

Variables	Level	Suspected cases (100)	Positive cases (7)	Negative cases (93)
Age	Less than 1 year	55	4	51
	More than 1 year	45	3	42
Gender	Male	51	6	45
	Female	49	1	48

The most prevalent isolated bacteria in CSF were coagulase-negative staphylococci, two isolates were *Staphylococcus sciuri* and one isolate was *Staphylococcus xylosus*, followed by two isolates of *Escherichia coli* and there was only one isolate for each of *Micrococcus luteus* and *Enterococcus cassiliflavus*, only one neonate (1%) had positive result for blood culture which was *Escherichia coli*, the same organism as the CSF culture Table 4.

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Table 4. Number and percentage of isolated bacteria from suspected meningitis in pediatric patients.

Body fluids	Bacterium	No. and (%) of positive isolates by culture and Vitek 2 System
CSF	*Escherichia coli*	2 (2%)
	Staphylococcus sciuri	2 (2%)
	Staphylococcus xylosus	1 (1%)
	Micrococcus luteus	1 (1%)
	Enterococcus cassiliflavus	1 (1%)
Blood	*Escherichia coli*	1 (1%)

Genotyping confirmation was carried out with a PCR based method involving specific and universal primers targeted against *uspA*, *16S rRNA* and universal *16S rRNA* genes. In this study, both isolates of *E. coli* from CSF as well as one isolate from blood were genotypically confirmed by *uspA* gene with an expected size of 884 bp, Fig. 1: Lane 2 from blood, 3 and 4 from CSF, *Staphylococcus sciuri* were genotypically confirmed by *16S rRNA* gene with expected size of 372 bp, Fig. 1: Lane 5 and 6. The results of molecular method were parallel with VITEK 2 Compact System identification for all 3 *E. coli* isolates, the *16S rRNA* gene PCR followed by amplicon sequencing identified two species of *Staphylococcus sciuri*, the same result as VITEK-2 Compact System, as shown in Table 5. While *Staphylococcus xylosus* was identified by VITEK-2 Compact System, whereas by PCR analysis, *Bacillus subtilis* were identified as shown in Table 5 and Fig. 1, Lane: 7. As well as *Micrococcus luteus* and *Enterococcus casseliflavus* identified by VITEK-2 Compact System, while by PCR analysis *Staphylococcus aureus* and *Bacillus subtilis* were identified, respectively as shown in Table 5 and Fig. 2, Lane 1 and 2, respectively.

Table 5. Comparison between VITEK-2 Compact System and molecular approach.

No.	VITEK-2 Compact System	% Identified	PCR assay	% Identified	Genes
1	*Staphylococcus sciuri*	97	*Staphylococcus sciuri*	100	*16S rRNA*
2	*Staphylococcus sciuri*	99	*Staphylococcus sciuri*	100	
3	*Staphylococcus xylosus*	99	*Bacillus subtilis*	98.45	
4	*Micrococcus luteus*	99	*Staphylococcus aureus*	92.39	Universal *16S rRNA*
5	*Enterococcus casseliflavus*	96	*Bacillus subtilis*	92.54	
6	*E. coli*	96	*E. coli*	100	*uspA*
7	*E. coli*	98	*E. coli*	100	*uspA*

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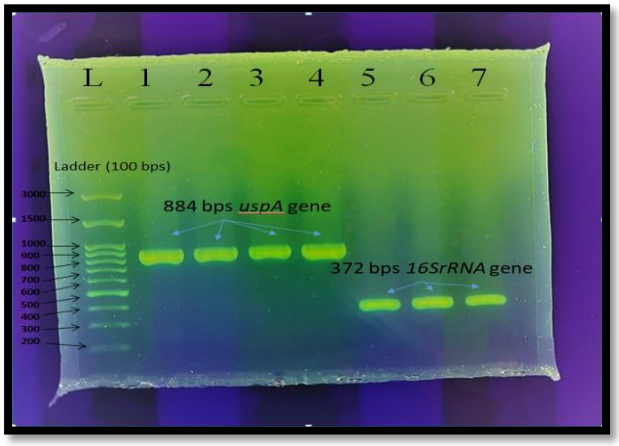

Figure 1. PCR product on agarose gel electrophoresis for the detection of *uspA* gene and *16S rRNA* genes. L: 100 bp DNA Ladder, Lane 1: Positive control *uspA* gene (884bp) (*E. coli* ATCC 25922), Lane 2: Positive isolate of *E. coli* from blood culture, Lane 3 & 4: Positive isolates of *E. coli* from CSF sample, Lane 5 and 6: Positive isolates of *Staphylococcus sciuri* and Lane 7: Positive isolate of *Bacillus subtilis*

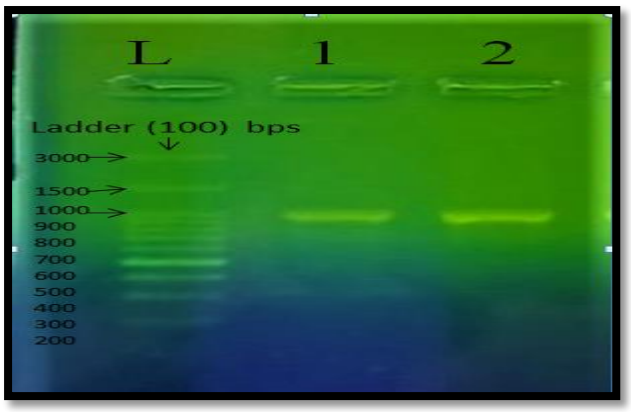

Figure 2. PCR product on agarose gel electrophoresis for the detection of *16S rRNA* gene. L: 100 bp DNA Ladder, Lane 1: Positive isolate of *Staphylococcus aureus* and Lane 2: Positive isolate for *Bacillus subtilis.*

In this investigation, isolated bacteria were exposed to several antibacterial to determine their sensitivity patterns for each microorganism as shown in Table 6. Meropenem has been shown to be totally resistant to both gram-positive and gram- negative bacteria. Among gram-negative bacteria, both isolated *E. coli* were resistant to cefotaxime, gentamycin, pencillin, ceftriaxone, rifampin, amoxicillin/clavulanic acid, ceftazidime, erythromycin, ampicillin, and clindamycin, while both isolates were sensitive to meropenem, imipenem, amikacin and tobramycin, and were intermediate sensitive to nitrofurantoin. One isolate was resistant to ciprofloxacin, azithromycin and trimethoprim/sulfamethoxazole while the other isolate was sensitive to ciprofloxacin, azithromycin and trimethoprim/sulfamethoxazole as in Table 6. This might be due to the antimicrobial drugs' increased use in the empirical treatment of meningitis and other illnesses.

In the current study both isolates of *Staphylococcus sciuri* were sensitive to tobramycin, trimethoprime, gentamycin, trimethoprim/sulfamethoxazole, meropenem and ofloxacin, they were resistant to ceftazidime, cefotaxime, bacitracin, pencillin, amoxicillin/clavulanic acid and optochin, and they were intermediate sensitive to clindamycin and erythromycin, one of the isolated was rifampin resistant and intermediate sensitive to vancomycin, while other isolate was vancomycin resistant and rifampin sensitive as in Table 6.

Whereas *Staphylococcus xylosus* was resistant to pencillin, ceftazidime, bacitracin, vancomycin, erythromycin, amoxicillin/clavulanic acid, optochin and rifampin, the isolate was sensitive to clindamycin, tobramycin, trimethoprime, gentamycin and meropenem as in Table 6.

Table 6, showed that *E. casseliflavus* was resistant to rifampin, ceftazidime, pencillin, optochin, clindamycin and bacitracin, while sensitive to trimethoprim, tobramycin, vancomycin, gentamycin, erythromycin, amoxicillin/clavulanic acid and meropenem.

Micrococcus luteus was resistant to erythromycin, penicillin and optochin; it was susceptible to amoxicillin/clavulanic acid, bacitracin, gentamycin, clindamycin, meropenem, rifampin, tobramycin, trimethoprim and vancomycin, and intermediately sensitive to ceftazidime as shown in Table 6.

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Table 6. Percentage of sensitivity and resistance of isolated bacteria to numerous antibiotics using disk diffusion method (Standard Kirby-Bauer).

Isolated bacteria	*Staphylococcus Sciuri*			*Staphylococcus Xylosus*			*Micrococcus luteus*			*Enterococcus casseliflavus*			*E. coli*		
Antibiotic's scientific name	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**
Amoxicillin/Clavulanic Acid	100	0	0	100	0	0	0	0	100	0	0	100	100	0	0
Azithromycin	-	-	-	-	-	-	-	-	-	-	-	-	50	0	50
Ampicillin	-	-	-	-	-	-	-	-	-	-	-	-	100	0	0
Amikacin	-	-	-	-	-	-	-	-	-	-	-	-	0	0	100
Bacitracin	100	0	0	100	0	0	0	0	100	100	0	0	-	-	-
Ceftazidime	100	0	0	100	0	0	0	100	0	100	0	0	100	0	0
Cefotaxime	100	0	0	-	-	-	-	-	-	-	-	-	100	0	0
Ceftriaxone	-	-	-	-	-	-	-	-	-	-	-	-	100	0	0
Ciprofloxacin	0	50	50	-	-	-	-	-	-	-	-	-	50	0	50
Clindamycin	0	100	0	0	0	100	0	0	100	100	0	0	100	0	0
Erythromycin	0	100	0	100	0	0	100	0	0	0	0	100	100	0	0
Gentamycin	0	0	100	0	0	100	0	0	100	0	0	100	100	0	0
Imipenem	100	0	0	100	0	0	-	-	-	-	-	-	0	0	100
Meropenem	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100
Nitrofurantoin	-	-	-	-	-	-	-	-	-	-	-	-	0	100	0
Ofloxacin	0	0	100	-	-	-	-	-	-	-	-	-	-	-	-
Optochin	100	0	0	100	0	0	100	0	0	100	0	0	-	-	-
Penicillin	100	0	0	100	0	0	100	0	0	0	0	100	100	0	0
Rifampin	50	50	0	100	0	0	0	0	100	100	0	0	100	0	0
Tobramycin	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100
Trimethoprim	0	0	100	0	0	100	0	0	100	0	0	100	50	0	
Trimethoprim/ Sulfamethoxazole	0	0	100	-	-	-	-	-	-	-	-	-	50	0	50
Vancomycin	50	0	50	100	0	0	0	0	100	0	0	100	-	-	-

R: resistant, I: Intermediate, S: Sensitive

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According to laboratory measures, the culture-positive group of CSFs had greater amounts of protein, leukocyte, and lower glucose than the culture-negative group of CSFs. The positive CSF culture and positive blood culture group had greater CRP levels than negative CSF culture and negative blood culture as seen in Table 7 and Fig. 3.

Table 3, indicated that the individuals with bacterial meningitis displayed (3/7) 42.8% of these patients had a CSF leukocyte count >1,000 cells/mm^3, and (4/7) 57.1% had a leukocyte count > 100 - 1,000 cell/mm^3 as shown in Fig. 3c.

Moreover, the results in Table 7, of laboratory findings of patients with bacterial meningitis showed lower glucose concentrations, high protein level, high leucocyte count and high level of CRP. In this study, a high relation was observed between positive bacterial culture and low glucose level, high protein level, high leukocyte count, and high CRP levels. From a statistical point of view, there was a significant difference between positive cultures and the parameters P<0.0001, as revealed in Figs. 3a, 3b, 3c and 3d.

Table 7. Laboratory findings of patients with bacterial meningitis

Patient no.	Sex	Age	Identified Bacteria	Glucose (mg/dl)	Protein (mg/dl)	Leukocyte count (Cells/mm^3)	CRP in CSF	Blood culture	CRP in serum
1	M	1.4 year	*Escherichia coli*	1	84.6	1100	24	-	12
2	M	2 months	*Escherichia coli*	1	189	2000	24	+	48
3	M	24 days	*Staphylococcus sciuri*	1	350	540	24	-	24
4	M	3 months	*Staphylococcus sciuri*	15	155	380	12	-	24
5	M	7 years	*Staphylococcus xylosus*	2.3	107	1160	48	-	96
6	F	2.4 year	*Micrococcus luteus*	16	87.3	300	0	-	0
7	M	3 months	*Enterococcus casseliflavus*	15.5	160.1	540	12	-	0

M: male; F: female.

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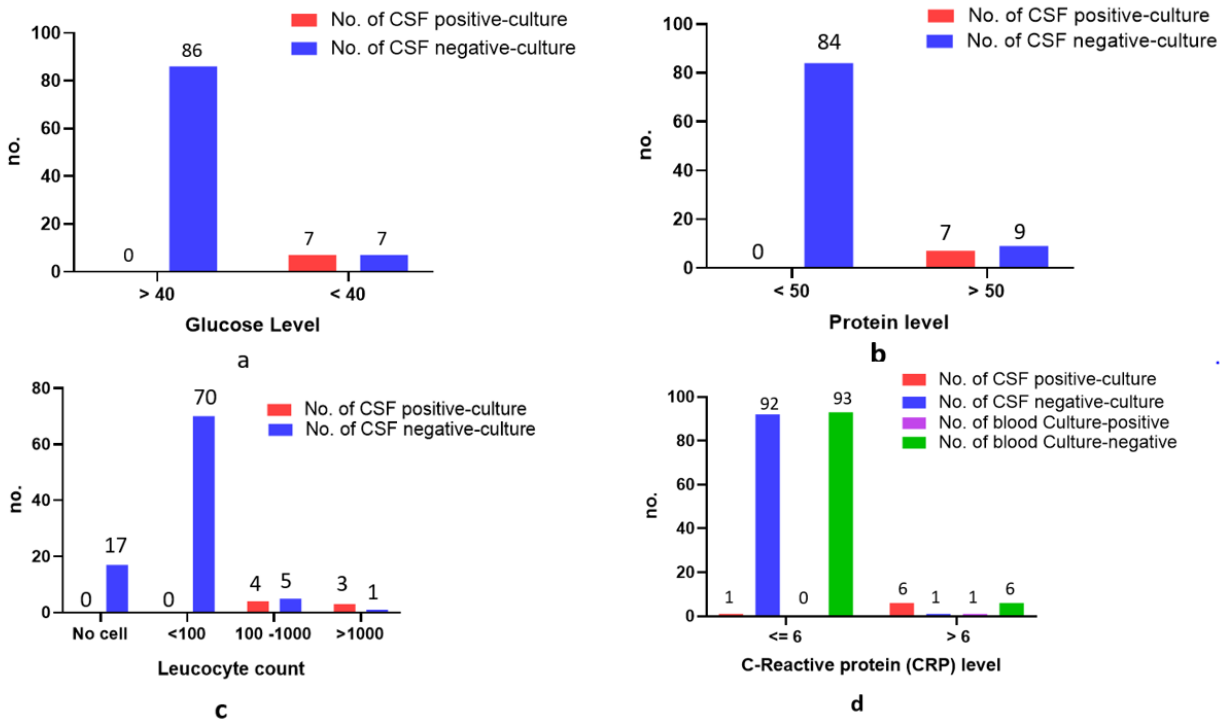

Figure 3. Relation between parameters and positive bacterial culture in CSF and Blood.

Discussion

Bacterial meningitis is one of the most serious health issues in children and infants. In this research found that the most prevalent organisms isolated from CSF were coagulase-negative staphylococci followed by *E. coli*. The current investigation supports Chang and colleagues' [26], findings that coagulase-negative staphylococci were the most prevalent microorganism in CSF cultures. Another research concluded that gram negative bacteria, most notably *Escherichia coli*, and gram positive bacteria accounted for around one-third of the microorganisms associated with coagulase-negative staphylococci [27]. In a study performed by Jeter and colleagues [28], indicated that *E. coli* was the most common bacteria causing meningitis.

One investigation found an instance of meningitis caused by *Micrococcus luteus* in a formerly healthy one year old infant [29]. In the other investigation, *Enterococcus casseliflavus* was not detected in CSF culture, and only one instance of the such bacterium was identified in a 77-year-old female as bacterial meningitis [30].

Staphylococcus xylosus, a commensal bacteria found in animals' mouth cavities, can cause severe infections. *Staphylococcus xylosus* was found in the CSF of a 9-year-old child who suffered a dog bite on his right forearm and thigh and was bleeding from the injured area [31]. Only one instance of *Staphylococcus sciuri* was evaluated in every two studies [32,26].

Gram positive bacteria were the most prevalent microorganisms identified in this investigation, which is consistent with previous findings [4]. Gram staining of CSF can help make an early preliminary etiologic diagnosis because CSF culture can take up to 48 hours. Its positive rates are dependent on the number of bacteria in the CSF, therefore the lack of the bacterium on gram stain does not rule out meningitis [9].

Bosshard and his colleagues [33], indicated that conventional identification resulted in *Nocardia brasiliensis*, *Bacillus* sp., *Actinomyces sp.*, *Microbacterium* and *Corynebacterium* sp., whereas sequence comparison with public databases resulted in 100% sequence identity with *Streptomyces albidoflavus*, *Paenibacillus* sp., *Actinobaculum* sp., *Corynebacterium mucifaciens* and *Propionibacterium acnes*, respectively.

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Antimicrobial resistance of *E. coli* exacerbates the prevalence of infections, particularly in newborns and mothers. *E. coli* related meningitis was discovered to be amoxicillin (80%) and rifampicin (77.5%) resistant, while sensitive to meropenem 92.5% and amikacin 97.5% [34]. In a study by Assegu Fenta and his colleagues [35], indicated that *E.coli* was resistant to penicillin, ceftriaxone, cefotaxime, and ciprofloxacin. A study conducted by Rasool [36], found that *E. coli* was sensitive to amikacin and ciprofloxacin while resistant to gentamycin and erythromycin. Another study found that *E.coli* was resistant to clindamycin, cefotaxime and vancomycin and sensitive to imipenem, and amikacin in Duhok/Iraq [37]. The resistance rates of *E. coli* to ceftriaxone and cefotaxime in this investigation were comparable to earlier Chinese studies [38,39]. The increased antibiotic resistance of *E. coli* is a major problem [40].

Marsou and colleagues [32], discovered that all 30 *S. sciuri* isolates were vancomycin and rifampin resistant. Tobramycin and gentamicin resistance was observed in five strains. Erythromycin resistance was found in eight strains. Another investigation found that rifampin, vancomycin, and amoxicillin/clavulanic acid were effective against all isolates of coagulase negative staphylococci including *S. sciuri* [26]. In a study by Singh and Jain [31], *Staphylococcus xylosus* was resistant to penicillin and erythromycin but responsive to gentamicin, ciprofloxacin, clindamycin, vancomycin and trimethoprim-sulpha-methoxazole.

In the study performed by Hassan and Bilal [41], *E. casseliflavus* was resistant to gentamicin, vancomycin, and erythromycin. In another research, *E. casseliflavus* was susceptible to penicillin, ampicillin, and imipenem; it was intermediately sensitive to vancomycin, trimethoprim-sulfamethoxazole, and ciprofloxacin; it was resistant to clindamycin and had high resistance to gentamicin and streptomycin [30].

The indicated laboratory criteria for bacterial meningitis were as follows: glucose level < 40 mg/dL, protein level > 50 mg/dL, and white blood cell count greater than 100 cells per mm^3 [42]. In this investigation, there was a significant difference between positive cultures and the parameters $P<0.0001$, as revealed in Fig. 3a, 3b, 3c and 3d.

C-reactive protein (CRP) is an indicator of inflammation, and its levels rise throughout bacterial infection. Researchers found that the CRP level in cerebrospinal fluid in meningitis patients was greater > 6mg/dl than in aseptic meningitis. CRP rises throughout inflammation in reply to monocytic mediators including IL-1 and IL-6 and has a consistent decay rate. The majority of the connection between CRP and the immunological response to microorganisms is assumed to entail CRP binding to phosphocholine (PCh) and stimulation of the classical complement pathway [43].

Studies performed by Dashti and coworkers [15] and Javadinia and coworkers [44], found that CRP levels were elevated in cases of bacterial meningitis which agree with the current study.

Martinot and colleagues [45] as well as Boskabadi and colleagues [46] have confirmed these findings, in which (3/7) 42.8% of patients with meningitis had a CSF leukocyte count >1,000 cells/mm^3 and (4/7) 57.1% had a leukocyte count > 100 - 1,000 /mm^3.

The results of biochemical analysis of patients with BM showed lower CSF glucose concentrations and high CSF protein according to this finding, the majority of researches found the same results [37]. The current investigation was supported by Wang and Zhu's [47] finding that CSF culture-positive bacterial meningitis had greater amounts of protein in CSF. Devi and coworkers [19] discovered that increased CSF protein occurs in 13% of meningitis patients and reduced CSF glucose occurs in 7.5%. In another study, an elevated quantity of protein in CSF was one of the poor prognostic variables in meningitis patients [48]. The rupture of the CSF-blood barrier results in a high CSF protein level in meningitis patients [49]. Bacterial metabolic activity causes a decrease in CSF glucose level, which is a common observation in instances of bacterial meningitis [50].

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Conclusion

In this study, it was determined that, in cases when gram-stained smears are negative, a combination of high CSF protein content, high WBC count, low glucose level, and increased CRP can predict bacterial meningitis until culture findings are available. When the gram staining test was negative, it might be because there were just a small number of bacteria in the CSF sample or because their population had declined as a result of prior antibiotic therapy.

On the other hand, the bacterium was not identified in a large number of cases, and after analyzing the results, the causes of infections may be related to viral meningitis. Besides that, Because of the small amount of specimen that could not be centrifuged to obtain sediment of the sample containing concentrated bacteria, the positive rate of CSF culture is remarkably low. Additionally, suboptimal storage and transportation conditions may have an impact on the positive rate of CSF culture. Meropenem and tobramycin have been shown to be 100% resistant to both gram-positive and gram-negative bacteria.

Staphylococcus sciuri, *Staphylococcus xylosus*, *Enterococcus casseliflavus*, and *Micrococcus luteus* were first identified as causing bacterial meningitis in Erbil, Iraq, in the current investigation.

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee in University of Salahaddin.

Authors' Contribution Statement

Both the authors planned this study and contributed to the interpretation of the data. N. O. H. conducted all the experiments and A. K. K. contributed to the revision of the draft and on proof reading. Both authors read and approved the final manuscript.

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الطرق التقليدية والجزيئية لتشخيص التهاب السحايا الجرثومي في مدينة أربيل ، العراق

نيكا عثمان حمه أمين، عادل كمال خضر

قسم الأحياء، كلية التربية، جامعة صلاح الدين، أربيل، العراق.

الخلاصة

يعد التهاب السحايا البكتيرى سبباً رئيسيا للمرض السحايا والوفاة عالمياً، والتشخيص المبكر لمسببات التهاب السحايا في الوقت المناسب امر بالغ الاهمية للرعاية الصحية السريرية والعامة، وكذلك مكافحة المرض. خلال الفترة من كانون الثاني 2021 الى شباط 2022، تم جمع مائة نموذج من سائل دماغي الشوكي وعينة الدم من الحالات المشتبه اصابتها بالتهاب السحايا التي تم ادخالها الى مستشفى رابرين التعليمي للاطفال في مدينة اربيل، العراق. تم اجراء التحاليل المزرعية و البايوكيميائية وتم تصديقها بواسطة التقنيات الجزيئية. اظهرت النتائج المزرعية ان 7% من العينات السائل الدماغي الشوكي وواحدة في نماذج الدم كانت موجبة. اكثر مسببات الامرض شيوعا التي تم العثور عليها من خلال الخصائص المزرعية و استخدام نظام فايتك 2 كانت *Stphylococcus sciuri* لنموذجين 2% ، *Staphylococcus xylosus* في نموذج واحد 1% ، *Escherichia coli* في نموذجين 2% ، *Micrococcus luteus* , *Enterococcus casseliflavus* والتي تم تسجيلهما لأول مرة كمسبب لالتهاب السحايا في مدينة اربيل، العراق. جميع العزلات تم تصديقها بواسطة تقنية PCR .تم فحص جميع العزلات المرضية بحثا عن حساسية هذه العزلات لبعض المضادات الحيوية، اظهرت النتائج ان جميع العزلات كانت مقاومة للمضاد الحيوي ميروبينيم و توبرامايسين بنسبة 100%، في حين اظهرت عزلة *E. coli* مقاومتها بنسبة 100% لكل من سيفوتاكسيم، جينتامايسين، بينيسيلين، سيفتريكسون، ريفامبيسين، اموكيسيلين/كلافولانك اسيد، سيفتازيديم، ايرثروميسين، امبيسيلين، وكليندااميسين الا انها كانت حساسة 100% لاميكاسين و اميبينيم، الا ان جميع عزلات الموجبة لصبغة غرام اظهرت مقاومتها و بنسبة 100% لاوبتوجين و كانت حساسة 100% الجينتامايسين و ترامیثوبريم. كما اوضحت النتائج ان في مرضى اتهاب السحايا الجرثومي تم ملاحظة ارتفاع كل من البروتين التفاعلي (>50 mg/dl) CRP وبروتين CSF(>50 mg/dl) وانخفاض في مستوى سكر الكلوكوزفي السائل الدماغي الشوكي(<40 mg/dl) و ارتفاع عدد الخلايا البيضاء (>100 cells/mm^3) في السائل الشوكي.

الكلمات المفتاحية: التهاب السحايا البكتيرى، عينة الدم، السائل النخاعي الشوكي، الخصائص المزرعية، تفاعل البوليميراز المتسلسل.