Isolation and Identification of *Staphylococcus* Spp. and Gram Negative in Meningitis Patients

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Abstract:

Introduction: Meningitis is known as a disease that Infect the central nervous system (CNS), it is mainly caused by bacteria, mycobacteria, fungi, viruses and parasites that cause disease and death, bacterial infections constitute the highest percentage of the other species, with (56.6%) and viruses (30.2%).

Aim: The present study aimed to isolate bacteria cause meningitis and identify these bacteria.

Materials and Methods: A total 248 clinical samples of cerebrospinal fluid (CSF) from meningitis patients were collected, for the period of July to October 2018. From the Child Protection Teaching Hospital in the Medical City Complex in Baghdad. All clinical samples were collected and cultured on blood agar, chocholate agar and MacConkey agar plates then incubated at 37˚C for 24 hours. All isolates were identificated depending on macroscopic, microscopic and biochemical tests (Oxidase, Catalase, Gelatinase, Haemolysin, growth at 15˚C, 45˚C, Novobiocin, coagulase for *Staphylococcus* spp.) and (Voges-Proskauer, Methyl red, Indol, Citrate, Urase, Kliglar, Catalase, Oxidase, for Gram negative bacteria) and definite with Vitek-2 compact.

Results: The results of the study showed growth of 42 samples, 7/16(44%) *S. epidermidis*, 3/16(19%) *S. hominis*, 3/16(19%) *S.haemolyticus*, 2/16(12%) *S.aureus*, 1/16(6%) *S.warneri*. Whereas the Gram negative bacteria include, 1(2%) isolate of *Pseudomonas putida*, *Escherichia coli*, *Klebsiella pneumonia*, and 2(4%) isolates *Burkholderia cepacia* and *Acinetobacter baumannii*, and 19(45%) isolates were others (Enterococcus faecium, streptococcus thoraltensis, *Kocuria kristinae*, *Leclerciaade carboxylata*, *Rothiadent ocariosa*, *Globicatella sulfidifaciens*, *Pantoea* spp., *Pantoeaag glomerans*).

Conclusion: Various species of bacteria appeared but Gram positive *Staphylococcus* spp. was more predominant.

Keywords: *Staphylococcus* spp., Gram-negative bacteria, Meningitis, Identification, Isolation.

Introduction:

Staphylococcus spp. are spherical, single, pairs or short chains of 3-4 cells and are irregular clusters in the form of grape clusters, (1.5 - 0.5) μm in diameter, it is Gram positive, nonmotile, capsule-forming or limited formation. Most *staphylococcus* strains grow with (10%) NaCl and temperature 18-40 °C, catalase positive and oxidase negative[(1)]. It exist naturally and mainly on the skin, glands and mucous membranes of warm-blooded animals, it can isolated from various sources including animal products (eg. meat, milk, cheese), from various environmental sources (soil, sand, dust, air, natural water, as well as clothing and stuff) [(2)]. Some types of *Staphylococcus* spp. as *S.aureus* are opportunistic caused by diseases such as boils, stomatosis, toxic lipolysis,

pneumonia, osteomyelitis, myocarditis, enteritis, mastitis, cystitis, prostatitis, cervicitis, meningitis, bacteremia, as well as internal toxins that cause food poisoning [3, 4]. These bacteria have many of the structures that produces virulence factors including, Teichoic acid (TA) is found on the surface of the Gram positive bacteria [5], that essential in the adherence, colonization and inflammation [6, 7], and has role in adhesion to fibronectin [8]. Intracellular matrix- binding protein (Embp) is a giant protein [9], which are also involved in adhesion and biofilm formation [9, 10], as well as possession of protein A [11]. Protein A is an important factor that protects bacteria from the immune system [12], and has roles in adhesion [13], and soft tissue [14]. There are many species of bacteria *Staphylococcus* spp. including *Staphylococcus aureus*, which possesses many virulence factors, including microbial surface component recognizing adhesive matrix molecules (MSCRAMMs) which acts on adhesion to cellular exogenous materials such as fibronectin, fibrinogen and collagen [11]. As well as having an enzyme hyaluronidase and Clumping factor [15]. Meningitis is known as a disease that Infect the central nervous system (CNS), it is mainly caused by bacteria, mycobacteria, fungi, viruses and parasites that cause disease and death, bacterial infections constitute the highest percentage of the other species, with (56.6%) and viruses (30.2%) [16]. Robertson *et al* [17] recorded infects 2,907,146 people a year with meningitis all over the world. Bhagat *et al* [18] noted that the symptoms of meningitis in neonates, including Tachycardia, increased to more than 160/minutes, or Bradycardia (less than 100/ minutes), respiratory symptoms such as snoring, respiratory cramps and rapid breathing (Tachypnea). Dashti *et al.* [19] noted that the clinical symptoms of meningitis are fever, bulging fontanels irritation or unknown sensations, alteration of unexplained consciousness, nuchal rigidity, vomiting, lack of clear awareness, persistent nausea and frequent episodes of fever.

Khan *et al* [20] pointed out that the chemical tests to be performed on the cerebrospinal fluid CSF sample are glucose content, which is less than or equal to 40 mg/dL, an increase in the level of protein to more than or equal to 60 mg/dL, and count the number of white blood cells (WBC count), which is more than or equal to 100 cells/mm^3. Therefore, the present study aimed to performing a study on meningitis patients and identifying the bacterial causes of this disease.

Materials and Methods:

The study included collecting 248 clinical samples of cerebrospinal fluid (CSF) from patients, attended Child Protection Teaching Hospital in the Medical City Complex in Baghdad for the period of July to October 2018. All clinical samples were collected and cultured on Blood agar, Chocholate agar and MacConkey agar plates then incubated at 37˚C for 24 hours. All isolates were identified depending on macroscopic, microscopic, biochemical tests (Oxidase, Catalase, Gelatinase, Haemolysin, growth at 15˚C, 45˚C, Novobiocin, Coagulase for *Staphylococcus* spp.) [21] and (Voges-proskauer, Methyl red, Indol, Citrate, Urase, Kliglar, Catalase, Oxidase, for Gram negative bacteria), and identified by with Vitek-2 compact.

Results and Discussion:

Two hundred forty eight clinical samples were collected from the Children's Hospital Complex of children with meningitis. Samples were cultured on Blood agar, Chocolate agar and MacConkey agar and incubated at 37°C for 24 hours. All isolates were identified depending on macroscopic, microscopic, biochemical tests and identified with Vitek-2 compact system. Some isolates appeared pink on MacConkey agar medium, and the others appeared as colorless depending on their fermentation and non-fermentation of lactose sugar. While the isolates appeared on blood agar and chocolate agar, white, creamy and gray. Under the microscope, they reacted negatively and positively with Gram stain where some of them appeared as red rods, and the other appeared with purple cocci respectively. Biochemical tests were performed; table (1) shows the emergence of all isolates *Staphylococcus* spp. gave negative for oxidase test and positive for catalase. *S.aureus* and *S.haemolyticus* gave positive for gelatinase test, except *S.warneri* was negative, while *S.epidermidis*, and *S.hominis* were varied. Some of *S.epidermidis* and *S.hominis* had ability to produce β haemolysin where the other isolates did not have the ability to produce γ haemolysin. *S.aureus* and *S.warneri* produced γ haemolysin, while *S.haemolyticus* produced β haemolysin. All of *Staphylococcus* spp. incapable to grow at 15˚C whereas they produced capable to grow at 45˚C. All *Staphylococcus* spp. isolates were sensitive for Novobiosin antibiotic test. In current study, table (2) shows the biochemical tests, for Gram negative bacteria: *Acinetobacter baumannii*, *Burkholderia cepacia*, and *Pseudomonas putida* were negative for the Voges-Proskauer, Methyl red, Indol, Urase, while were varied for Citrate. *Klesiella pneumonia* isolates were negative for the Indole test, whereas Methyl red and Urase positive, while were varied for Citrate. *Escherichia coli* were indol positive and varied for Citrate. All isolates were not produced CO_2 and H_2S. All isolates non-glucose fermenting except *Klesiella pneumonia* and *Escherichia coli* are fermenting. All isolates were positive for catalase. For the purpose of diagnosing bacterial isolates of the Cerebrospinal fluid (C S F) using vitek- 2 compact through a series of chemical tests. The results in figure (1) showed *S. epidermidis* where 7/16(43.75%), whereas *S. hominis* and *S.haemolyticus* where recorded 3/16(18.75%), *S.aureus* 2/16(12.5%), S.*warneri* 1/16(6.25%). In figure (2) *Staphylococcus* spp. showed 16(38%), whereas the gram negative bacteria include, 1(2.38%) isolate of *Pseudomonas putida, Escherichia coli, Klebsiella pneumonia*, and 2(4.76%) isolates *Burkholderia cepacia* and *Acinetobacter baumannii*, and 19(45.24%) isolates were others (*Enterococcus faecium, Streptococcus thoraltensis, Kocuria kristinae, Leclerciaade carboxylata, Rothiadento cariosa, Globicatella sulfidifaciens, Pantoea* spp., *Pantoea agglomerans*). As shown in figure (2). Reta et al., (2016) [22] reported that the rate of isolated (CONs) bacteria from neonatal cerebrospinal fluid was (4%) and (5%) for *S. aureus* bacteria with illustration that the isolated rate difference according to neonatal weight and infection type. In contrast Rhie et al., (2017) [23] informed higher rate of *S.*

aureus about (27.1%) in infant less than 3 months, 20.5 in infant 3-59 months and (26.8%) in children with 5 and older. Khan et al., (2017) [20] reported that higher infected with CONs bacteria as a result of hospital infections and (90%) of this bacteria was resistant to Oxacillin antibiotic, with a suggestion that the increased infection with *S. aureus* because of resistance to Oxacillin antibiotic along with lack of vaccine; in the other hand, the rate infected with *Haemophilus influenza* was decreased because of vaccine availability. The variety in isolated rate of this study with other previous studies might be due to sample type and possibly antibiotic take, also the ability of some laboratories in isolation diagnosis.

Table(1): Biochemical tests for *Staphylococcus* spp.

Bacteria species	Oxidase	Catalase	Gelatinase	Haemolysin	Temperature		Novobiocin	Coagulase
					15˚C	45˚C		
S.epidermidis	-	+	- ، +	γ،β	-	-,+	S	-
S.aureus	-	+	+	γ	-	+	S	+
S.warneri	-	+	-	γ	-	+	S	-
S.hominis	-	+	+,-	γ، β	-	+	S	-
S.haemolyticus	-	+	+	β	-	-,+	S	-

(+): Positive, (-): negative, (γ):gamma haemolysis, (β):beta haemolysis, (S):sensitive >12mm.

Table (2): Biochemical tests for Gram negative bacteria.

Bacterial species	MR-VP		Indol	Citrate	Urase	Kliglar iron agar				Catalase	Oxidase
	Voges-Proskauer	Methyl red				Slant	Bottom	CO_2	H_2S		
Acinetobacter baumannii	-	-	-	±	-	K	K	-	-	+	-
Burkholderia cepacia	-	-	-	±	-	K	K	-	-	+	-
Klesiella pneumoniae	/	+	-	±	+	A	A	-	-	+	-
Pseudomonas putida	-	-	-	±	-	K	K	-	-	+	/
Escherichia coli	/	/	+	/	-	A	A	-	-	/	-

(-): negative reaction, (+): positive reaction, (±): various, (A): Acidic, (K): Alkaline, (/): not data

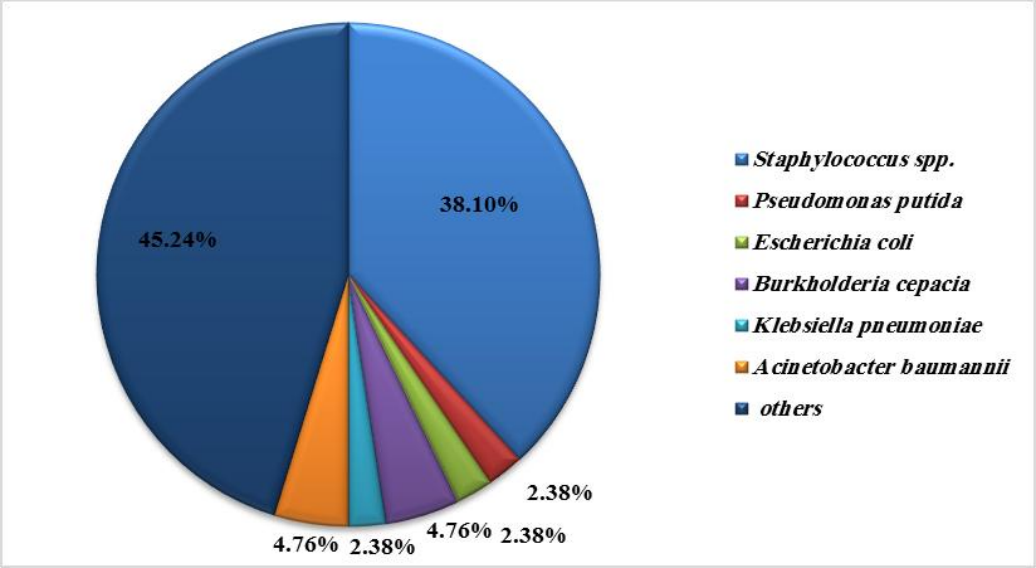

Figure (1): Percentage of *Staphylococcus* spp. isolates.

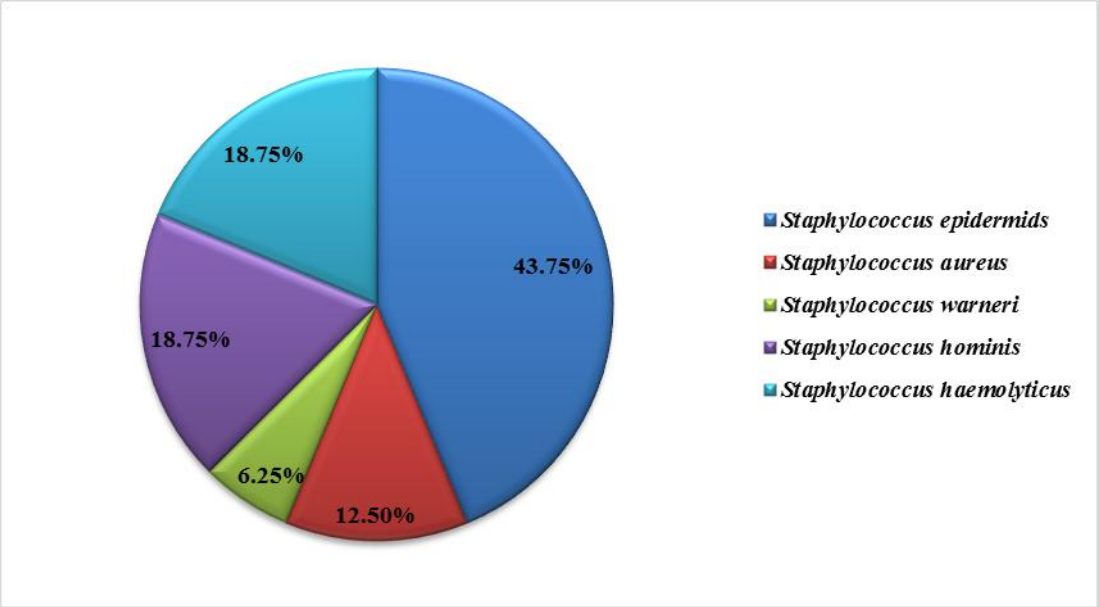

Figure (2): Percentage of bacterial isolates isolated from meningeal fluid.

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