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Retrospective analysis of bacterial agents in central nervous system infections and antibiotic resistance: A nine years evaluation at a university hospital

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Abstract

The follow-up of bacterial agents and antibiotic resistance profiles that cause central nervous system (CNS) infections has guided the choice of life-saving empirical treatment against these infections. This study aimed to evaluate the bacterial agents isolated from cerebrospinal fluid (CSF) samples and their antibiotic susceptibility for nine years with the suspicion of CNS infection. We retrospectively analyzed the results of the patients sent CSF samples for bacteriological diagnosis with the suspicion of CNS infection between August 2012 and April 2021. The isolated bacterial species were identified using conventional methods, biochemical tests, and various commercial identification systems. Microorganisms were isolated in 121 (14.85%) of the 815 CSF samples examined. The most commonly isolated agents are methicillin-resistant coagulase-negative *Staphylococci* (n: 59; 48.76%) among Gram-positive and *Klebsiella* spp. (n: 12; 9.92%) among Gram-negative. The follow-up of bacteria isolated from CNS infections, which can progress rapidly and cause serious complications, and the changes in antibiotic susceptibility over the years are known as a guide in regulating appropriate treatment. Our results may contribute to the selection of antibiotics that can be used especially in cases where urgent empirical treatment is required.

Keywords: antimicrobial resistance, bacterial agents, central nervous system, infection, cerebrospinal fluid

1. Introduction

Central nervous system (CNS) infections such as meningitis, encephalitis, and brain abscesses are associated with high mortality and morbidity; for this reason, prompt diagnosis and effective treatment are crucial. CNS infections, which can be caused by various microorganisms such as viruses, bacteria, parasites, and fungi, can progress faster than other infectious diseases and can cause permanent damage or death in a short time (1). Bacterial pathogens are more common among the causative agents of CNS infections (2).

Most CNS infections are community-acquired, but they may develop related to health care due to trauma, use of cerebrospinal fluid shunts, and external drains in some patients. In community-acquired bacterial CNS infections, organisms commonly isolated include *Streptococcus pneumoniae, Neisseria meningitidis,* and *Haemophilus influenzae* (3). However, frequently detected agents may change over the years depending on risk factors such as age, geographical differences, seasonal changes, vaccination status, genetic structure, and socioeconomic conditions (4-6).

Accurate diagnosis of bacterial agents and monitoring of antibiotic resistance patterns over the years are life-saving and guide empirical treatment selection. In other words, empirical treatment depends on regional patterns of antibiotic resistance of common pathogens. This study aimed to evaluate the bacterial agents isolated from cerebrospinal fluid (CSF) samples and their antibiotic susceptibility for nine years with the suspicion of CNS infection.

2. Materials and Methods

2.1. Study Design and Patients

We retrospectively analyzed the results of the patients who were sent CSF samples for bacteriological diagnosis with the suspicion of CNS infection between August 2012 and April 2021. In the CSF samples sent to the laboratory at different times, the same bacteria on the same patient and with the same antibiotic sensitivity were accepted as only one isolate. Other isolates were not evaluated in this study.

2.2. Culture conditions and antibiotic susceptibility tests

All CSF samples admitted to the laboratory were evaluated after inoculation on 5% sheep blood agar, chocolate agar, and MacConkey agar media and incubated for 18-36 hours at 37°C. The identification of microorganisms from specimens that have shown growth detected by conventional methods was achieved by analyzing colony structure, growth characteristics, and conducting biochemical tests. In addition, all CSF samples sent to the laboratory were inoculated into automatic blood culture system bottles (BACTEC BD, United States). Gram staining and subculture passages were performed to identify the agent from the blood culture bottle with a positive signal.

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Antibiotic susceptibility tests of isolated bacteria on Mueller-Hinton agar were performed with the Kirby-Bauer disc diffusion method using commercial antibiotic discs (Oxoid, England) according to Clinical Laboratory Standards Institute (CLSI) (before 2016) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) (after 2016) criteria (7, 8).

Phoenix (BD, United States) and MALDI-TOF MS (Bruker, Germany) commercial identification and antibiotic susceptibility systems were also used in doubtful identifications.

2.3. Statistical Analyses

The patient data was evaluated with IBM SPSS 20.0 (IBM Corp., Armonk, NY, USA) package program and Microsoft Excel version 2013. Descriptive statistics were used to define the age of the patients, and frequencies (n) and percentages (%) were used to describe microbiological culture test results.

3. Results

Microorganisms were isolated in 121 (14.85%) of the 815 CSF samples examined. Sixty-eight (56.2%) were isolated from male, and 53 (43.8%) were isolated from female patients. These patients' ages ranged from 0 to 76 years, and the mean age and SD were 23.92 ± 26.21 years.

The most commonly isolated agents are methicillin-resistant coagulase-negative *Staphylococci* (MRCoNS) (n:59; 48.76%) among Gram-positive and *Klebsiella* spp. (n:12; 9.92%) among Gram-negative (Table 1).

Table 1. Distribution of bacteria (n:121) isolated from CSF samples

Bacteria	Number (n)	%
Gram positive		
Coagulase-negative staphylococci	59	48.76
Staphylococcus aureus	9	7.44
Streptococcus spp.	9	7.44
Enterococcus spp.	6	4.96
Gram negative		
Klebsiella spp.	12	9.92
Acinetobacter spp.	10	8.26
Pseudomonas spp.	6	4.96
Enterobacter spp.	5	4.13
Escherichia coli	2	1.65
Serratia marcescens	1	0.83
Stenotrophomonas maltophilia	1	0.83
Chryseobacterium indologenes	1	0.83

The distribution of isolated microorganisms by years is given in Fig. 1. Table 2 presents the distribution of culture positivity in CSF samples based on the clinics to which they were sent for laboratory analysis. An examination of the requesting clinics regarding culture positivity revealed that most of the samples were sent from the Neurosurgery clinic (n:31; 25.62%), followed by the Pediatrics and Anesthesia and Reanimation clinics (Table 2).

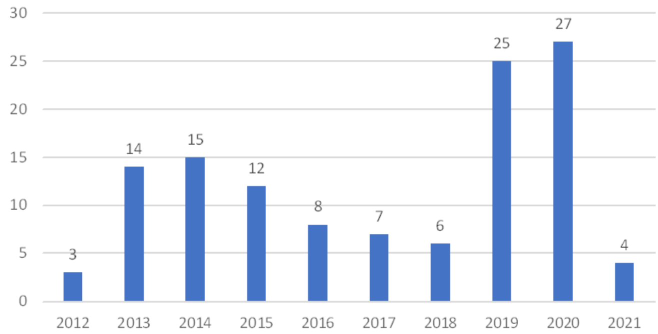

Fig.1. Distribution of isolated bacteria years

Table 2. Distribution of CSF samples with growth in culture according to clinics

Clinics	n	%
Neurosurgery	31	25,62
Pediatrics	29	23,97
Anesthesia and Reanimation	23	19,01
Neurology	14	11,57
Newborn	13	10,74
Infectious Diseases and Clinical Microbiology	3	2,48
Radiology	3	2,48
Others*	5	4,13

* Orthopedics and Traumatology, Medical Oncology, Emergency Medicine

It was determined that 99 of the 121 patients with culture positivity in CSF samples were found samples from Turkish citizens, 12 were Libyan citizens, 3 were Iraqi citizens, 2 were Azerbaijani citizens, 2 were Moldovan citizens, and one of these CSF samples taken from Georgia, Kazakhstan, and Russian citizens. Antibiotic susceptibilities of isolated strains are given in Table 3.

Table 3. Antibiotic resistance rates of isolated bacteria from CSF samples

Bacteria	Antibiotic resistance* (%)														
	DA	AN	GN	TOB	LZD	AMP	TZP	AMC	CAZ	FEP	MEM	IMP	CIP	LEV	SXT
Coagulase-negative staphylococci (59)	43.4	17.9	47.6	57.6	2.7								64.7	62.7	37.5
Klebsiella spp. (12)		25	83.3			100	70	70		90	41.7	36.4	63.6	54.5	0
Acinetobacter baumannii complex (10)		40	60	30			60		80	60	60	60	60	50	0
Staphylococcus aureus (9)	11.1	33.3	22.2	33.3		44.4							22.2	0	0
Streptococcus spp. (9)	11.1					33.3				11.1					
Enterococcus spp. (6)			50		0	16.7							100	100	
Pseudomonas spp. (6)		0	16.7	0			16.7		16.7	0	40	20	20	20	
Enterobacter spp. (5)		0	20			100	0	80	0	0	0	0	20	0	0
Escherichia coli (2)		0	0			50	0	0	0	0	0	0	0	0	0
Serratia marcescens (1)		0	0			100		100							0
Stenotrophomonas maltophilia (1)														0	
Chryseobacterium indologenes (1)		100	0	100			100		100	100	100	100	0	0	0

* DA: clindamycin; AN: amikacin; GN: gentamicin; TOB: tobramycin; LZD: linezolid; AMP: ampicillin; PRP: piperacillin-tazobactam; AMC: amoxicillin-clavulanic acid; CAZ: ceftazidime; FEP: cefepime; MEM: meropenem; IMP: imipenem; CIP: ciprofloxacin; LEV: levofloxacin; SXT: trimethoprim-sulfamethoxazole

4. Discussion

Despite the development of antibacterial agents and modern microbiological methods used in the differential diagnosis of the agents, bacterial CNS infections are serious infections that cause morbidity and mortality worldwide (1, 9). These life-threatening infections require immediate antimicrobial therapy, prompt identification of the pathogen, and early initiation of appropriate antimicrobial therapy are essential to improve clinical outcomes (10, 11).

Significant differences exist in the spectrum of pathogens isolated from cerebrospinal fluid cultures in developed and developing countries. While in developed countries, Group B streptococci (GBS), *Escherichia coli,* and *Listeria monocytogenes* were generally found as important organisms; there were bacteria such as *Klebsiella* spp., *E. coli,* and *Streptococcus pneumoniae* as well as Group B streptococci in developing countries (12).

In our study, the most common agents were methicillin-resistant coagulase-negative staphylococci among Gram-positive bacteria and *Klebsiella* spp. among Gram-negative bacteria. These were followed by *Acinetobacter baumannii* complex, *Staphylococcus aureus,* and *Streptococcus* spp., respectively.

In the study conducted by Durand et al. (13) with 493 cases of acute bacterial meningitis in adults in the USA, the rate of hospital-acquired infection was 40%. Except for *H. influenzae,* Gram-negative bacteria have been reported to cause 33% of hospital-acquired meningitis. Frequent use of invasive medical supplies and contamination of respiratory support equipment are factors that increase the likelihood of nosocomial infections (14).

Klebsiella spp. and *Acinetobacter baumannii* complex, with the extraordinary spreading ability of the most common pathogens isolated from CSF samples, leads to mainly considering nosocomial infections. The high number of Gram-negative bacteria among the causative agents indicates that hospital-acquired infections are more common than community-acquired infections. In addition, when the distribution of CSF samples with culture positivity according to clinics was examined, Neurosurgery ranks first, followed by Pediatrics and Anesthesia, respectively. It can be predicted that the higher number of microorganisms reproducing from CSF in these units may be due to the gradual increase in invasive interventions and the prolongation of hospital stays with the developing technology.

Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) recommended that specific vaccines against *S. pneumoniae, Neisseria meningitidis*, and

Haemophilus influenzae type b be added to national immunization programs; thus, a decrease in cases caused by these microorganisms has been observed (15-18). In our study, *N. meningitidis* and *H. influenzae* could not be isolated in any case during the nine-year period, and *S. pneumoniae* was isolated in only one case. This finding is consistent with studies conducted in developed countries where meningitis vaccines are administered (19, 20). We think this situation may be due to the introduction of *H. influenzae* type b and conjugated pneumococcal vaccines into routine vaccination in our country after 2006.

In some reports conducted in our country and worldwide, the detection rates of bacterial agents in CSF were between 5.3% and 31.5% (13, 14, 21-23). Our study isolated bacteria in 121 (14.85%) of 815 CSF samples. This rate may vary depending on the development level of the countries, age, the way the agent is taken, routine vaccination programs, geographical location, and treatment or operation.

The distribution of isolated microorganisms varies from year to year. The lowest distribution by year appears in 2012 and 2021. The examined periods included short times such as 4 months in 2012 and 3 months in 2021, which may explain why the lowest distribution of isolated microorganisms occurred during these years. However, no specific reason for the increase in the number of microorganisms isolated in 2019 and 2020 has been identified, and it is considered a spontaneous increase.

In conclusion, the data obtained from this study showed that the most common agents isolated from CSF samples are Coagulase Negative Staphylococci, *Klebsiella* spp., and *Acinetobacter* spp. We believe the periodic determination of the microorganisms causing CNS infection and their resistance rates may be significant in controlling nosocomial infections and guiding empirical treatment regulation. Therefore, we think that routinely analyzing collected clinical laboratory data in monitoring antimicrobial resistance will benefit the review of treatment guidelines.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: A.I.T., Design: A.I.T., S.S., Data Collection or Processing: A.I.T., M.D., Analysis or Interpretation: A.I.T., S.S., M.D., Literature Search: S.S., Writing: S.S., M.D.

Ethical Statement

Ethics committee approval is not required for this study.

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