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Detection of *Enterobacteriaceae* in cerebrospinal fluid of neonates with meningitis from tertiary care hospitals

Z. NUREEN[1], Z. BASIT[1], I. MUSARAT[2], R. AYESHA[1], S. FALK[1], F. YUMNA[1], S. ABID[1], T. AZIZ[3], M. GHANI[3], A. METAB[4], T.H. ALBEKAIRI[4], F.A. ABDULLAH[4]

[1]Institute of Molecular Biology and Biotechnology, The University of Lahore, Defense Road Lahore, Punjab, Pakistan
[2]Lymphatics and Regenerative Surgery Laboratory, Obrien Institute and St Vincent's Institute, Melbourne, Australia
[3]Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan
[4]Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

Abstract. – OBJECTIVE: This study aimed to determine the bacteriological profile of childhood acute bacterial meningitis in Pakistan.

PATIENTS AND METHODS: The study included a total of 100 children aged between 1 month and 5 years, who were admitted with a diagnosis of meningitis based on clinical findings and positive cerebrospinal fluid (CSF) tests. Out of the 100 CSF samples collected, 21 isolates were confirmed to contain *Enterobacteriaceae*. The most prevalent *Enterobacteriaceae* species were *Pseudomonas* (n=8, 38.09%), *Klebsiella* (n=4, 19.04%), *E. coli* (n=4, 19.04%), and *Acinetobacter* (n=4, 19.04%), while *Citrobacter* (n=1, 4.76%) was less common. Antibiotic susceptibility patterns were analyzed for these isolates.

RESULTS: *Pseudomonas* (n=8) exhibited 25% resistance to cefepime and 38% resistance to imipenem. *Klebsiella* (n=4) showed 75% resistance to imipenem. *Acinetobacter* (n=4) demonstrated 50% resistance to imipenem, along with varying resistance to cefepime, amikacin, ciprofloxacin, and gentamicin. *E. coli* (n=4) showed 0% resistance to imipenem and amikacin. However, *Citrobacter* (n=1) showed 0% resistance to ciprofloxacin, aztreonam, gentamicin, amikacin, levofloxacin, and cefepime. Acute bacterial meningitis primarily affects children under 1 year of age.

CONCLUSIONS: CSF culture revealed that Gram-negative bacteria, specifically *Pseudomonas spp.*, were the predominant pathogens in this family based on Pakistani data.

Key Words:
Bacterial meningitis, Cerebrospinal fluid (CSF), Drug resistance (DR), *Enterobacteriaceae*.

Introduction

Meningitis, described as an inflammation of the meninges, is caused by various pathogens and is associated with a high rate of morbidity and mortality[1]. The World Health Organization (WHO) categorizes meningitis as one of the top five neonatal infections worldwide[2]. Bacterial meningitis is linked to high morbidity and mortality rates. The death rate in affected neonates ranges up to 10%, and 20-50% of survivors develop other diseases[3]. Infections due to multi-drug resistant Gram-negative bacteria, primarily *Enterobacteriaceae*, are a major concern in newborns[4-8]. Neonates are at high risk of sepsis and meningitis due to their immature cellular and humoral immunity. The organisms found in meningitis are the same as those in neonatal sepsis. Like neonatal sepsis, meningitis is also characterized by early-onset and late-onset forms[9].

Risk factors for neonatal meningitis are similar to those for neonatal sepsis, including maternal factors, virulence factors of pathogens, and neonatal host factors. Premature infants and infants with very low birth weight (VBLW) are at high risk of meningitis[6]. The disease exhibits nonspecific signs and symptoms; however, reported signs and symptoms include lethargy, difficulty in breathing, and poor vision. Neurological signs involve seizures, twitching of the face, changes in consciousness, and gaze deviation[6].

Diagnosis of bacterial meningitis depends on the results of routine examination of cerebrospinal fluid (CSF). Characteristics such as an in-

Corresponding Author: Tariq Aziz, MD; e-mail: iwockd@gmail.com

creased protein level in CSF, a high neutrophil count in CSF, and low glucose levels are initial indications of septic meningitis[7]. Gram-negative bacteria causing meningitis include *Escherichia coli*, *Klebsiella*, *Proteus*, *Citrobacter* spp, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Salmonella*, and *Enterobacter* spp[6,8].

Antibiotic therapy is necessary to remove pathogens from CSF as early as possible and is used for both gram-positive and gram-negative bacteria until the pathogen is confirmed[9]. Initial antimicrobial therapy for early meningitis should be administered in combination with aminoglycosides and ampicillin. Third-generation cephalosporins should be administered when the cause of meningitis by Gram-negative bacteria is confirmed[10,11]. It is recommended to use carbapenem in combination with aminoglycosides due to some cephalosporin and carbapenem-resistant strains of Gram-negative bacteria[12]. When choosing an appropriate antimicrobial, consider the resistance pattern of possible microorganisms in the community.

To determine the disease pattern, etiological agents, and outcome of childhood acute bacterial meningitis in the *Enterobacteriaceae* family.

The aim of this study is to analyze the bacteriological profile of the *Enterobacteriaceae* family in childhood acute bacterial meningitis.

Patients and Methods

The study was conducted in compliance with the local institutional biosafety and bioethics committee of the University of Lahore, Lahore, Pakistan, at different tertiary care hospitals in Lahore. Informed consent was obtained from the parents of neonates before sampling.

Collection of CSF Samples from Neonates

A total of 100 CSF samples from clinically confirmed neonates with meningitis were collected. All samples were immediately transported to the laboratory for processing following the Clinical Laboratory Standard Institute (CLSI) protocol and standard guidelines[9].

Microbiological Analysis of CSF Samples from Neonates

The CSF samples from neonates were directly inoculated on blood, MacConkey, and chocolate agar (Oxoid Ltd) and incubated at 37°C for 24 hours. The plates were observed for the growth of pathogens. Plates with no growth were further incubated for 24 hours. Gram-negative bacteria isolated on blood agar were further sub-cultured on MacConkey agar [(Oxoid Ltd) Basingstoke, Hampshire, UK]. A phenotypic examination was conducted by observing morphology, culture characteristics, and performing biochemical tests. Gram-negative rods were confirmed using the triple sugar iron, citrate, oxidase, and motility tests, and further investigated using the API 20E kit method[13].

Identification of E. coli, Pseudomonas, Klebsiella, Citrobacter, Acinetobacter, and Other Organisms

Medial identification Nutrient Agar (NA): all isolates appeared as colorless small colonies except *Pseudomonas,* which appeared as green, color-swarming growth. Blood agar (BA): after 16-24 hours of incubation in 5-10% CO_2 at 35-37°C, Gram-negative rods, with a diameter of 2-3 mm, displayed low, convex, grey, mucoid, and swarming growth in *Klebsiella, Citrobacter*, and *Acinetobacter* spp. MacConkey (MAC) agar: After 16-24 hours of incubation in the air at 35-37°C, Gram-negative rods appeared colorless (lactose non-fermenting) in *Klebsiella, Pseudomonas*, and pink in color in *Acinetobacter* and *E. coli*. Cystine-lactose-electrolyte deficient (CLED) agar with bromothymol blue (CLED B) or Andrade's indicator (CLED A): After 16-24 hours of incubation in the air at 35-37°C, *E. coli* and *Acinetobacter* appeared yellow (lactose fermenting), and *Klebsiella, Citrobacter*, and *Pseudomonas* appeared blue (lactose non-fermenting). Desoxycholate citrate agar (DCA): *E. coli* appeared pink (lactose fermenting) or colorless (lactose non-fermenting), while other Enterobacteriaceae appeared blackish in the butt. Xylose-lysine-desoxycholate agar (XLD): *Acinetobacter, E. coli,* and *Citrobacter* (sucrose fermenting) appeared yellow (xylose, lactose, or sucrose fermenting), and others were pink in color. Thiosulphate-citrate-bile salt (TCBS) agar: Gram-negative rods appeared yellow (sucrose fermenting) in *Citrobacter*, while others were bluish in color.

Biochemical Identification

Triple sugar iron: *E. coli* and *Acinetobacter* appeared yellowish due to lactose fermenting in the slanted part of the test tube, while *Citrobacter* appeared yellowish in the slant and blackish in the butt, and *Pseudomonas* appeared reddish in color in the entire test tube. Simon Citrate test: *E. coli*

tested negative for the Simon Citrate test, whereas *Acinetobacter*, *Klebsiella*, *Pseudomonas*, and *Citrobacter* tested positive for this test. Oxidase: all *Enterobacteriaceae* tested negative for the oxidase test. After morphological and biochemical identification, *Enterobacteriaceae* were further investigated using the API 20E Kit method.

Antibiotic Susceptibility Testing of Confirmed Isolates

Antibiotic susceptibility testing of isolated Enterobacteriaceae was conducted using the Kirby Bauer disc diffusion method following Clinical Laboratory Standard Institute (CLSI) guidelines. A total of 14 clinically important antibiotic discs [(Oxoid Ltd) Basingstoke, Hampshire, UK] were applied, including tazobactam (110 µg), ciprofloxacin (5 µg), aztreonam (30 µg), gentamicin (10 µg), clarithromycin (15 µg), cefotaxime (30 µg), amikacin (30 µg), ceftazidime (30 µg), levofloxacin (5 µg), Cefepime (30 µg), Onaxabid (5 µg), imipenem (10 µg), tetracycline (30 µg), and cefoxitin (30 µg)[17]. Inhibition zones were measured according to CLSI guidelines.

Results

Frequency of Bacteria in Neonatal Meningitis

A total of 100 samples of CSF were analyzed, among which only 21 samples were confirmed for the presence of *Enterobacteriaceae*. Out of 21 positive samples *Pseudomonas* (38.09%) was most prevalent, followed by *Klebsiella* (19.04%), *E. coli* (19.04%), and *Acinetobacter* (19.04%). The least prevalent pathogen found was *Citrobacter* (4.76%) (Figure 1).

Antimicrobial Susceptibility Pattern of Gram-Negative Bacteria Isolated from Neonatal Meningitis

The antimicrobial susceptibility pattern showed that the most effective drug against *Pseudomonas* (n=8) was Cefepime, which showed 25% resistance, and imipenem with 38%. *Klebsiella* (n=4) showed 75% resistance to imipenem. *Acinetobacter* (n=4) showed 50% resistance to imipenem, followed by cefepime, amikacin, ciprofloxacin, and gentamicin. *E. coli* (n=4) showed 0% resistance to imipenem and amikacin. However, *Citrobacter* (n=1) showed 0% resistance to ciprofloxacin, aztreonam, gentamicin, amikacin, levofloxacin, and cefepime (Table I; Figure 2).

Discussion

Neonatal meningitis is considered one of the most dangerous infection[14]. It is a serious disease with substantial mortality and morbidity[2]. Contemporary to the development of intensive care, neonatal meningitis is still a lethal disease[15-17]. Neonatal meningitis is characterized by non-specific signs and symptoms. Approximately 90% of bacterial meningitis occurs in neonates during their first five years of life. This disease may result in about 12% mortality, and the rate may increase up to 30%. Effective management of a disease requires information on its cause, risk factors, and antimicrobial susceptibility. Group B *Streptococcus* (GBS) and *Escherichia Coli* (*E-coli*) have been reported as the major pathogens of bacterial meningitis in young infants[18] in various developed countries, while in the developing world, microbiology of bacterial meningitis in neonates

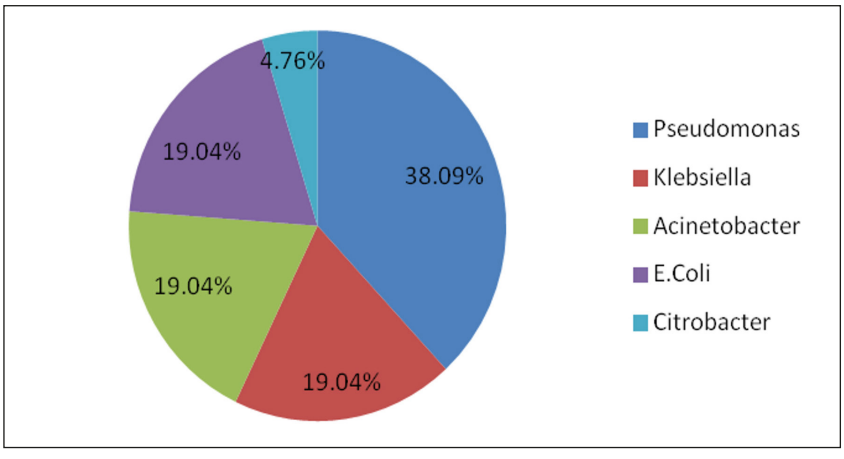

Figure 1. Frequency of isolated bacteria from clinically isolated cerebrospinal fluid sample. The most prominent candidate is *Pseudomonas* spp 38.09% and *Acinetobacter* spp, *E. coli,* and *Klebsiella* spp 19.04%, and the least one isolate were *Citrobacter* 4.76%.

Table I. Antimicrobial resistance pattern of Gram-negative bacteria in neonates.

	Pseudomonas spp (n=08)	*Klebsiella* (n=04)	*Acinetobacter* (n=04)	*E. coli* (n=04)	*Citrobacter* (n=01)
Tazobactam	100%	100%	75%	75%	100%
Ciproflocin	63%	100%	50%	75%	0%
Aztreonam	100%	100%	100%	100%	0%
Gentamicin	75%	100%	50%	50%	0%
Colistin	100%	100%	75%	100%	100%
Cefepime	100%	100%	100%	100%	100%
Amikacin	100%	100%	50%	0%	0%
Ceftazidime	100%	100%	100%	100%	100%
Levofloxcin	50%	100%	100%	50%	0%
Cefetamet	25%	100%	50%	25%	0%
Tobramycin	100%	100%	75%	100%	100%
Imipenem	38%	75%	50%	0%	100%
Tetracycline	75%	100%	100%	100%	100%
Cefoxitin	50%	100%	100%	50%	100%

differs geographically[19-21]. A study[17] supported by WHO in four African countries demonstrated that *Klebsiella Pneumoniae* and *E. coli* were among the most prevalent causative pathogen in cases of neonatal meningitis. *E-coli* was the most common isolated bacterium in cases of neonatal meningitis followed by other pathogens, including *Staphylococcus epidermidis*, *K. pneumonia,* and GBS in different studies[21-23] performed in central and western provinces of China.

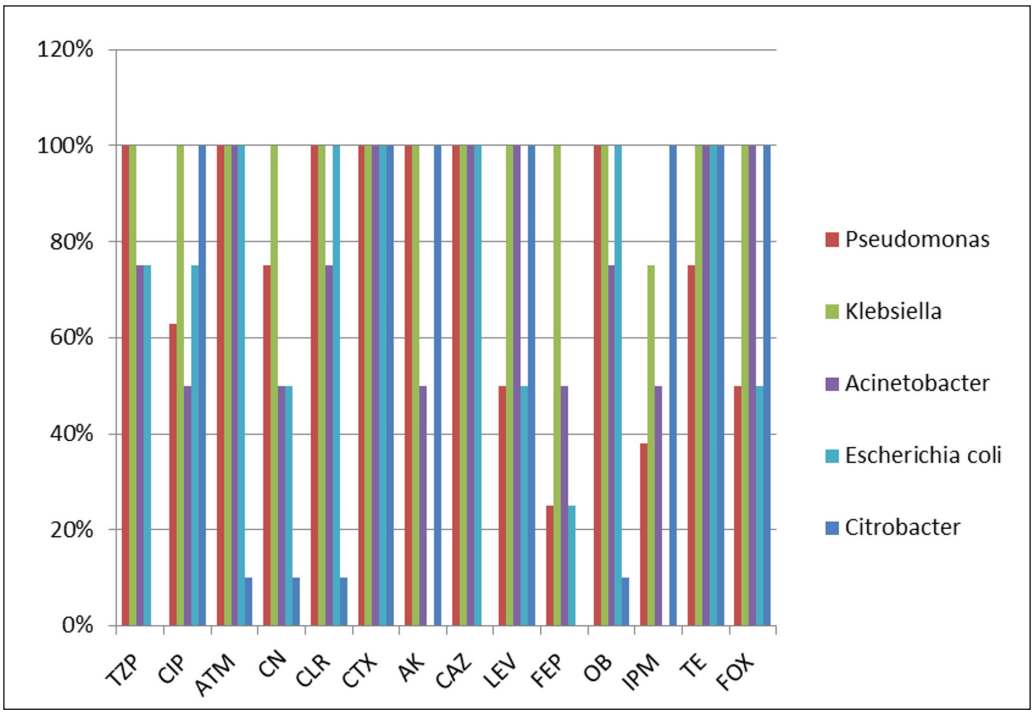

Figure 2. Antimicrobial pattern of gram-negative bacteria (*Enteriobacteraece* family) with different antibiotic tazobactam (110 µg), ciprofloxacin (5 µg), aztreonam (30 µg), gentamicin (10 µg), clarithromycin (15 µg), cefotaxime (30 µg), amikacin (30 µg), ceftazidime (30 µg), levofloxacin (5 µg), cefepime (30 µg), tobramycin (5 µg), imipenem (10 µg), tetracycline (30 µg), and cefoxitin (30 µg).

The common pathogens found in two-thirds of cases of neonatal meningitis are *E. coli* and *S. agalactia*[20,21]. Other studies[23-24] in western Sweden and China reported *E. coli* as the major pathogen as compared to other Gram-negative bacteria. However, in the present study, we found *Pseudomonas,* a highly frequent pathogen involved in causing neonatal meningitis. A study from India[17] reported a high prevalence of *Acinetobacter baumannii* (n=12) followed by *Klebsiella* (n=8) and *Pseudomonas* (n=6). We found that the most prevalent pathogens were *Pseudomonas* (38.09%), *Acinetobacter baumannii* (19.04%), and *Klebsiella* (19.04%) in this study. The most effective antibiotic against *E. coli* was aminoglycosides, imipenem, and amikacin, and the most effective cephalosporin was cefoxitin[5]. However, in our study, the effective antibiotics against Gram-negative bacteria were cefepime, imipenem, amikacin, ciprofloxacin, aztreonam, gentamicin, cefepime, and levofloxacin.

The present study revealed Gram-negative bacteria as a major cause of neonatal meningitis. Risk factors for neonatal meningitis are maternal factors, host factors, prematurity, low birth weight, and mode of delivery. In conclusion, bacterial meningitis is frequently present in Pakistan and shows antimicrobial resistance to clinically used antibiotics.

Conclusions

The study demonstrates that *Pseudomonas* spp exhibit more resistance to imipenem and cefepime antibiotics, while *Citrobacter* and *E. coli* showed no resistance to these antibiotics. From the results of cerebrospinal Fluid (CSF) culture, it was evident that *Pseudomonas* spp was prominent among all.

Availability of Data and Materials

All the data generated in this research study has been included in this manuscript.

Ethics Approval

Ethical Approval for this study was approved by the Institute of Molecular Biology and Biotechnology, The University of Lahore, under Ref No.: CRIMM/23/students/35, dated 15/09/2022.

Informed Consent

Informed consent was obtained from the parents of neonates before sampling.

Authors' Contributions

Conceptualization: Nureen; methodology, Basit.; software, Musarat; validation, Ayesha; formal analysis, Falak investigation, Abid; resources, Yumna; data curation, Metab writing-original draft preparation, Thamer.; writing-review and editing, Abdullah; visualization, Mustajab supervision, Tariq.; project administration, Abid; funding acquisition, Tariq.

ORCID ID

Abid Sarwar: 0000-0003-2105-7201
Tariq Aziz: 0000-0003-0905-8076

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Conflict of Interest

The authors declare no conflict of interest.

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