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To determine the prevalence and phenotype of ESBLs producing gram-negative isolates from cases of neonatal sepsis that occurred in Indore.

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Abstract

Background: This study's objectives are to identify and determine the prevalence of ESBLs producing gram-negative isolates from cases of neonatal sepsis that occurred in Indore, Madhya Pradesh; and also, to investigate the detection of antibiotic susceptibility pattern of gram-negative bacilli isolated in blood culture of neonatal sepsis. **Methods:** In terms of its methodology, the research can be categorized as an experiment that also consists of a descriptive component. Both of these components are included in the investigation. After receiving approval on an ethical level from the Institutional Ethical Committee of the Index Medical College Hospital & Research Centre (IMCH&RC), which can be found in the sentence before this one, it was carried. Inclusion criteria: Neonates under 60 days old with a clinical history of sepsis were admitted to the Index Medical College Hospital & Research Centre in Indore for blood cultures. Neonatal blood was drawn. The blood was cultured for further examination. The study included inborn and out born infants who were admitted to the IMCH and RC's Neonatal Intensive Care Unit (NICU) with suspected clinical features of sepsis at admission or developed such features after admission for other reasons. The study included infants admitted to the NICU and other IMCH and RC departments. The infants were given antibiotics even though it was unclear. **Results:** 50% of the GNB isolates were positive for ESBL. ESBL positivity was significantly more prevalent (>50%) in E. coli, Klebsiella spp., and Enterobacter spp. than in Acinetobacter spp. (1%). Similarly, 89% of E. coli, Klebsiella spp., and Enterobacter spp.were MDR, with Acinetobacter spp. There was a variation in susceptibility status according to specific bacterial genus and antimicrobial agent, but in general, fifty five percent of the GNB isolates that were tested showed resistance to the antimicrobials that were included in the first-line AST panel. **Conclusion:** The present study concludes that MDR bacteria, ESBL, and carbapenemase-encoding AMR genes in GNB make patient treatment difficult.

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Introduction:

Antibiotic resistance is responsible for 700,000 deaths worldwide each year [1,2]. Regrettably, if no immediate and effective action is taken, the death toll could rise to 10 million annually by the year 2050. The onset of neonatal sepsis can be broken down into two categories: early onset sepsis, which is also abbreviated as EOS, and late onset sepsis (LOS). These two categories can be used interchangeably. LOS disease is caused by bacteria picked up after delivery, whereas EOS disease is primarily caused by bacteria picked up before and during delivery. This distinction is important from a clinical standpoint because EOS disease is primarily caused by bacteria picked up before and during delivery (nosocomial or community sources). In spite of this, there is not a lot of consensuses on the appropriate age limits to apply across the board of the research that has been carried out. Very few studies make a distinction between very early onset sepsis VEOS (which occurs within 24 hours), EOS (which occurs between 24 hours and six days), and LOS (which occurs more than six days) (which occurs more than six days) [4,5]. The onset of symptoms at an age greater than 30 days is used to define very late onset sepsis [4]. It is possible for the pathogens that cause neonatal infections to change over time. Additionally, the pattern of antibiotic susceptibility that these pathogens exhibit can differ from one country to the next.

Neonatal sepsis is a leading cause of death all over the world, particularly in developing countries [5]. Developing countries account for the majority of these deaths. It is classified as either EOS, in which the first signs of infection appear within the first 72 hours after birth, or LOS, in which the first signs of infection appear more than 72 hours after birth. Both categories are subdivided further into subtypes. The EOS form is the most typical one [6,7]. In fifty percent of all cases, the death toll from sepsis is caused by sepsis that was acquired while the patient was in a hospital. This form of sepsis is more severe and displays a higher level of antibiotic resistance in comparison to the type of sepsis that was already present in the patient when they were admitted to the hospital [8-21].

This study's objectives are to identify and determine the prevalence of ESBLs producing gram-negative isolates from cases of neonatal sepsis that occurred in Indore, Madhya Pradesh; and also, to investigate the detection of antibiotic susceptibility pattern of gram-negative bacilli isolated in blood culture of neonatal sepsis.

Materials & Methods:

In terms of its methodology, the research can be categorized as an experiment that also consists of a descriptive component. Both of these components are included in the investigation. After receiving approval on an ethical level from the Institutional Ethical Committee of the Index Medical College Hospital & Research Centre (IMCH&RC), which can be found in the sentence before this one, it was carried.

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Subjects Criteria: Inclusion criteria: Neonates under 60 days old with a clinical history of sepsis were admitted to the Index Medical College Hospital & Research Centre in Indore for blood cultures. Neonatal blood was drawn. The blood was cultured for further examination. The study included inborn and out born infants who were admitted to the IMCH and RC's Neonatal Intensive Care Unit (NICU) with suspected clinical features of sepsis at admission or developed such features after admission for other reasons. The study included infants admitted to the NICU and other IMCH and RC departments. The infants were given antibiotics even though it was unclear.

The standard one-milliliter blood samples were collected sterilely and immediately placed in a sterile blood culture bottle with Brain Heart Infusion Broth. Collecting followed standard procedures. The blood culture bottle was taken to Indore's IMCH&RC microbiology lab after registration and labelling. It stayed until examined. After receiving the sample, this happened next. Culture procedures followed. Culture triumphed. This study required blood samples from neonates older than 60 days and without clinical evidence of sepsis. This study excluded infants with a history of prolonged rupture of the mother's membranes or labor, congenital anomalies, acute bilirubin, encephalopathy, perinatal asphyxia, meconium aspiration syndrome, or congenital anomalies. Congenital anomalies prevented infants from participating.

Sample Collection: The 1 ml blood samples were collected aseptically in accordance with neonate collecting procedures and immediately transferred to a sterile 30 ml blood culture bottle containing Brain Heart Infusion Broth (BHI) from the Hi Media Laboratory in Mumbai. Immediately after sample collection. After sampling, this was done immediately. The blood culture bottle was taken to the Indore IMCH&RC microbiology lab after registration and labelling. It stayed until examined. After receiving the sample, this happened next. Culture cultivation involved the following steps [10,11]. The incubator was set to 37 degrees Celsius after placing the culture bottle inside. Incubating the blood took five to seven days. The sample's growth, turbidity, hemolysis of red cells, gas bubble formation, and discrete colony clot formation were monitored daily. This information speeds up the presumptive diagnosis of a positive broth culture. After incubation, BHI broth on blood agar (BA; HiMedia, M073) and Mac Conkey agar were used for subculture (MA; HiMedia, M081). MA and BA plates were kept aerobic for 24 hours in a 37°C incubator. Candle jars formed the incubator [10].

Bacterial isolation and identification:

Conventional microbiological methods were used to identify pure isolates from subculture plates. These methods studied colony morphology, Gram staining reactions, and biochemical properties. Catalase and oxidase tests, Hugh Leifson's oxidative fermentative agar, sugar fermentation media, indole, methyl red, voges proskauer, simon's citrate, triple sugar iron agar, and Christensen's [10-19].

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Antibiotic resistance testing. The Clinical and Laboratory Standards Institute recommended using a modified Kirby-Bauer disc diffusion method to assess isolate antibiotic susceptibility. This determined which antibiotics would be most effective against the isolates (CLSI).

Antibiotic susceptibility:

Antibiotics such as ampicillin (10 g), amoxycillin (30 g), amoxiclav (20/acid 10g/disk), cefotaxime (30 g/disk), ceftazidime (30 g/disk), ceftazidime clavulanic acid (30/10 g/disk), ceftriaxone (30 g/disk), and cefaperazone (75 g/pipe) were utilised in this investigation. Himedia in India was contacted for the purpose of making a purchase of the entire assortment of antibiotic discs that were used in the susceptibility test. This was done in order for us to be able to carry out the test. E. coli ATCC 25923, Pseudomonas aeruginosa ATCC 27853, and Klebsiella pneumoniae ATCC 700603 were the reference strains that were used for quality control during the biochemical tests and the tests to determine the sensitivity of the bacteria to antibiotics. These strains were obtained from the American Type Culture Collection (ATCC). The American Type Culture Collection was consulted in order to acquire these strains (ATCC).

b) Controlling Quality: The quality of the prepared media was examined by putting one plate from each batch through a sterility test and a performance evaluation. Purity plates were utilized to ensure that the inoculation that was utilized for the biochemical tests was of the highest possible quality and to determine whether or not the biochemical tests were carried out in an aseptic environment. Both S. aureus ATCC 700,699 (MRSA) and S. aureus ATCC 29,213 (MSSA) were utilized as part of the quality control process.

Statistical Analysis:

All the obtained data will be entered into SPSS (version 22.0) and was analyzed. Descriptive as well as comparison of antibiotic susceptibility, Phenotytic and genotypic characterization of ESBL was done between ESBL producing gram negative bacilli and Non- ESBL producing negative bacilli.

Results:

Table 1: Gram negative bacteria isolated from blood samples obtained from different departments.

Gram negative bacteria profile	**Number**	**Percentage (%)**
E. Coli	94	34
Klebsiella spp.	70	25
Enterobacter spp.	63	23
Acinetobacter spp.	49	18
Departments of GNB isolation (N=276)		
NICU	140	50
Gynecology	38	14
Medical	30	11

PICU	22	8
ESBL positivity status of GNB (276)		
NO	135	49
YES	141	51

Table 3: The phenotypic status of ESBL positivity and Multi-drug resistant (MDR) of bacteria isolated from blood samples.

Bacterial pathogens	**ESBL-negative**		**ESBL-Positive**		**Non-MDR**		**MDR**	
	n	**%**	**n**	**%**	**N**	**%**	**n**	**%**
E.Coli (n=94)	42	45	52	55	8	12	46	88
Klebsiella spp. (n=70)	28	39	42	61	5	9	56	92
Enterobacter spp. (n=63)	24	37	39	63	1	2	38	98
Klebsiella spp. (n=49)	48	99	1	1	0	0	1	100

GNB distribution by the departments of isolation: The majority of GNB isolates were found in blood samples taken from neonatal patients who visited the departments including the neonatal intensive care unit (NICU) (50 percent). When the specific departmental origin of each four GNB were analyzed, it was discovered that 57 percent of Enterobacter spp. were isolated from patients who were admitted to the emergency department. In a similar vein, the neonates that were admitted to the PICU department had a prevalence of 28 % of K. pneumoniae in their systems. Anti-bacterial susceptibility testing of gram-negative bacteria isolated in the present study.
The modified Kirby-Bauer disc diffusion method [10-19] was used for the AST. The direct colony suspension method was used to prepare a suspension of bacterial inoculum, in which a few isolated bacterial colonies from an overnight culture on nutrient agar (Mast group Ltd., UK) were suspended in a glass tube containing sterile normal saline. A McFarland densitometer (DEN-1, Grant bio, Grant instruments, UK) was used to measure bacterial turbidity, which was adjusted to a reading of 0.5. The 0.5 McFarland bacterial suspension was then plated with a sterile cotton swab on Muller Hinton Agar (MHA) (Becton Dickinson and Company, USA) to create a confluent bacterial lawn. The antimicrobial discs were aseptically placed on the MHA plate with a minimum of 24 mm space between the disc centers. For the bacterial

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AST, the antimicrobial discs (Mast group Ltd., UK) shown in table 2.1 were applied to the MHA plate. Not all antimicrobials were tested initially for the bacterial isolates. The isolates were first tested for the first-line panel of antimicrobials. Only isolates that tested non-susceptible to all agents in the first-line panel were tested for second-line antimicrobial agents (Table 2.1). The MHA plates were incubated for 18 hours at 352°C. MHA plates inoculated with E. coli ATCC strain 259220 and K. pneumoniae ATCC strain 700603 were used as positive controls in each batch of AST. Following the specified incubation period, the sizes of the zone of inhibition around each antimicrobial disc were measured and interpreted using the zone size breakpoints recommended by the Clinical Laboratory Standards Institute (CLSI) [20-29]. The hospital followed the most recent CLSI guidelines on zone size breakpoints available in the respective years when interpreting AST results of bacterial isolates.

Tests to see if extended-spectrum beta-lactamase and carbapenemase are present:

It was hypothesized that GNB isolates that exhibited resistance (zone of inhibition of ≤ 22 mm for Enterobacteriaceae and ≤ 14 mm for Acinetobacter spp.) or intermediate susceptibility were responsible for the production of ESBLs (zone of inhibition 23–25 mm for Enterobacteriaceae and 15–22 mm for Acinetobacter spp.). 50% of the GNB isolates were positive for ESBL. ESBL positivity was significantly more prevalent (>50%) in E. coli, Klebsiella spp., and Enterobacter spp. than in Acinetobacter spp. (1%). Similarly, 89% of E. coli, Klebsiella spp., and Enterobacter spp.were MDR, with Acinetobacter spp.

AST results of isolated GNB bacteria: There was a variation in susceptibility status according to specific bacterial genus and antimicrobial agent, but in general, fifty five percent of the GNB isolates that were tested showed resistance to the antimicrobials that were included in the first-line AST panel. When it came to the first-line antimicrobial agents, less than six percent of the Enterobacteriaceae GNB that were tested were susceptible to cefotaxime, less than fourteen percent were susceptible to ciprofloxacin, less than thirty percent were susceptible to cotrimoxazole, less than forty-seven percent were susceptible to amikacin, and less than forty-three percent were susceptible to chloramphenicol, with the exception of E. coli. In the case of Acinetobacter spp., the percentage of isolates that were resistant to cefotaxime was only 2 %, whereas the percentage of isolates that were resistant to ciprofloxacin, cotrimoxazole, and amikacin was approximately 28 %. The percentage of Enterobacteriaceae GNB that were susceptible to the second-line antimicrobials that were tested ranged from less than twenty-one percent to sixty-one percent for meropenem and colistin, respectively, but less than nineteen percent were susceptible to piperacillin/tazobactam. In the case of Acinetobacter spp., less than fifteen percent of the isolates were sensitive to meropenem and piperacillin/tazobactam, whereas eighty-one percent of the isolates were sensitive to colistin.

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Positive phenotypic tests: Combination disc agar diffusion was utilized to perform a phenotypic confirmation test for the presence of ESBL [10-28]. This test was used to determine whether ESBL was present. A bacterial inoculum with a turbidity of 0.5 McFarland was prepared and inoculated on two MHA plates (Becton Dickinson and Company, USA) according to the protocol described in the methods section 2.6.2. This was done to obtain a semi-confluent bacterial lawn. Ceftazidime (30 g), ceftazidime (30 g) with clavulanate (10 g), cefotaxime (30 g), and cefotaxime (30 g) with clavulanate (10 g) were the four antimicrobial discs that were placed on the MHA plate to conduct the ESBL test (D62C, Mast Group Ltd., UK). Cefpodoxime (10 g), cefpodoxime (10 g) with ESBL-inhibitor, cefpodoxime (10 g) with AmpC-inhibitor, and cefpodoxime (10 g) with ESBL-AmpC-inhibitors (D68C, Mast Group Ltd.) were the four antimicrobial discs that were used to detect ESBL and/or AmpC production on the second inoculated plate. A strain of E. coli ATCC number 259220 and a strain of K. pneumoniae ATCC number 700603 were also included. At a temperature of 352 degrees Celsius, the MHA plates were kept warm for 18 hours. It was possible to determine the dimensions of the inhibition zone. In order to determine whether or not an isolate was positive for ESBL, the size of the zone of inhibition in a -lactamase inhibitor-supplemented disc was compared to the size of the respective -lactam alone. If the difference was 5 millimeters, the isolate was determined to be ESBL positive.

Discussion:

This study's objectives are to identify and determine the prevalence of ESBLs producing gram-negative isolates from cases of neonatal sepsis that occurred in Indore, Madhya Pradesh; and also, to investigate the detection of antibiotic susceptibility pattern of gram-negative bacilli isolated in blood culture of neonatal sepsis. In this study, 50% of GNB isolates tested positive for ESBL. E. coli, Klebsiella spp., and Enterobacter spp. had more ESBL genes (>50%) than Acinetobacter (1%). Similarly, 316 of 380 Acinetobacter spp., 648/717 E. coli, 488/541 Klebsiella, and 510/515 Enterobacter were MDR. This study found 50% ESBL positivity, higher than other studies. Other studies found MDR to be common in GNB. This study found a much higher ESBL positive prevalence. A study on community-acquired E. coli found 24% (48/200) positive for ESBL and 78% (156/200) positive for MDR [26]. Multiple-antibiotic-resistant E. coli strains were studied. In 2014, another hospital-based study examined urine isolates from National Kidney Centre hemodialysis patients suspected of UTIs. This study found that 27% (18/67) of E. coli had ESBL and 97% (92/95) of Enterobacteriaceae bacteria (mostly Klebsiella spp. and E. coli) were MDR [27]. Acinetobacter species had 81% (314/382) MDR, according to this study. In other hospital intensive care unit studies [28-30], clinical isolates of Acinetobacter spp. were also multidrug resistant (greater than 80 percent). In particular, 85 percent (47 out of 55) of Acinetobacter spp. isolated from tracheal aspirates of intensive care unit patients admitted to a tertiary care neuro-hospital between 2011 and 2012 were MDR

[28]. After MDR Acinetobacter spp. were isolated from tracheal aspirates of patients with COVID-19, this was discovered. In a different study [29], 95% (59/62) of Acinetobacter spp. isolated from lower respiratory tract specimens in a tertiary hospital's microbiology lab were MDR. However, my 1% ESBL prevalence for Acinetobacter spp. was significantly lower than the 12–13% reported by other studies on isolates from lower respiratory tract specimens of ICU-admitted patients in tertiary hospitals [28-30]. A study on A. baumannii isolated from tracheal aspirates of patients admitted to an intensive care unit in a tertiary hospital in Dhaka, Bangladesh, found that all 25 isolates tested negative for an ESBL phenotypic confirmatory test [31]. A. baumannii from Bangladeshi patients' tracheal aspirates was studied. This study matches mine. 79% of Acinetobacter spp. (1,060/1,347), 71% of Klebsiella (3,048/4,312), and 54% of E. coli (1,510/2,798) isolated from South Asian infant blood samples were MDR [32]. 42% (127/304) of K. pneumoniae, 33% (98/298) of E. coli from several Indian hospitals, and 16% (179/1,114) of enteric and non-enteric GNB from Bangladesh were ESBL positive [33]. ESBL positivity was highest in K. pneumoniae (42%, 127/304). 42% of Indian K. pneumoniae and 33% of E. coli samples tested positive for ESBL.

Conclusion:

MDR bacteria, ESBL, and carbapenemase-encoding AMR genes in GNB make patient treatment difficult. In settings with limited resources, suboptimal infection prevention and control (IPC) and inadequate antimicrobial usage policies, along with a high burden of GNB with transferable AMR genes, create an ideal environment for multidrug-resistant pathogens to emerge and spread.

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