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To determine the genotype of gram-negative isolates from cases of neonatal sepsis that occurred in Indore.

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Abstract

Background: This present study 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 genotypic characterization of ESBL producing gram negative bacilli isolated in blood culture of neonatal sepsis. **Methods:** The research is experimental and descriptive. Both are investigated. It was carried out after the Index Medical College Hospital & Research Centre (IMCH&RC) Institutional Ethical Committee approved it. Eligibility: The Index Medical College Hospital & Research Centre in Indore performed blood cultures on newborns with sepsis. Drawing neonatal blood. Cultured blood was tested. The study included inborn and out born infants admitted to the IMCH and RC's Neonatal Intensive Care Unit (NICU) with suspected clinical features of sepsis at admission or after admission for other reasons. NICU and IMCH/RC infants were studied. Despite uncertainty, the infants received antibiotics. **Results:** The detection of each bla_{OXA}, $bla_{\text{CTX-M}}$, and bla_{TEM} ESBL genes was significantly associated with an ESBL phenotypes. The strength of association varied by the specific ESBL genes, with the odds ratio (OR) of 1.4 (95% CI: 1.2-1.6, p<0.05) for bla_{OXA}, 3.6 (95% CI: 2.8-3.8, p<0.001) for bla_{TEM}, and 14.7 (95% CI: 11.4-18.8, p<0.001) for bla_{CTXM} in the Fisher's test. **Conclusion:** The findings of this study indicated that there was a high likelihood for GNB to be MDR and ESBL positive whenever any of the blaTEM, blaCTXM, or blaOXA genes were identified. In addition, the identification of the blaCTXM genes provided strong evidence that the GNB were resistant to the beta-lactam antimicrobials with an extended spectrum of activity.

Introduction:

Both the adult and pediatric populations in India are susceptible to bacterial bloodstream infections (BSIs), which can lead to an acute illness that is not well-defined other than a fever [1]. This is a prevalent public health problem in India. There is a high incidence of BSIs caused by drug-resistant organisms in both hospitals and communities [2]. In addition to this, the utilization of antimicrobials is something that can be found in

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extremely high rates in both hospitals and communities [3]. If we keep utilising antimicrobials in a manner that is not appropriate, there is a chance that the burden of antimicrobial resistance will increase even further [4-5]. Therefore, just like the other countries in South Asia, India is potentially responsible for a sizeable portion of the burden that is associated with antimicrobial resistance globally [6-8]. However, in this day and age of continually shifting bacteriological profiles and rising rates of antimicrobial resistance, the appropriateness of this kind of treatment, which consists of trial and error, is being called into question [5, 9, 10]. Before establishing guidelines for empirical therapy, it is important to have a basic understanding of the common organisms that can cause neonatal sepsis in a given region as well as the antibiotic sensitivity pattern of those organisms. This will allow for a more informed decision regarding which antibiotics should be used to treat the infection.

Hence, 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 genotypic characterization of ESBL producing 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. 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.

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Evidence of ESBL production at the genotypic level being found:

Using the lysis method, gram-negative bacterial cells that were shown to produce ESBL phenotypically were subjected to genomic DNA extraction. In order to extract the genomic DNA, this method was utilized. The bacterial genomic DNA extraction kit was utilized to its full potential in order to ensure the successful completion of the extraction (HiMedia, Mumbai). The comparison between the DNA samples and the uncut Lambda DNA was carried out with the assistance of the software known as Gel Doc (Biorad). After running the DNA samples through an agarose gel with a concentration of 0.8%, they were stained with ethidium bromide to visualize their patterns. Not only did the findings provide evidence that DNA was present, but they also provided information regarding the quantity of DNA that was there. A polymerase chain reaction (PCR) analysis was performed on the DNA sample that had previously been extracted in order to determine the presence or absence of the blaSHV, blaTEM, and blaCTX M genes. This was done so that it could be determined whether or not these genes were present. You will need approximately 2.5 microliters of PCR buffer (without MgCl2), 2.5 microliters of 25 mM MgCl2, 0.2 microliters of 200 mM deoxyribonucleotide triphosphate, 1 microliter of each forward and reverse primer (10–12 pmole), and 0.1 microliter of 5U Taq polymerase in order to carry out a single reaction (HiMedia). In addition to that, one microliter of the genomic DNA that had been extracted was added to the mixture that contained the reactants while the reaction was going on. In order to meet the requirements, the volume was increased to 25 ul by using deionized water as the solvent. This allowed for all of the prerequisites to be satisfied. Electrophoresis was carried out on a 1.5% agarose gel so that a comparison could be made between the amplified PCR products and the 100 bp ladder that was supplied by HiMedia. This was done in order to determine the nature of the relationship between the two. After the gels were stained with ethidium bromide, the results could be viewed using either an ultraviolet trans-illuminator or a Gel Doc instrument. The gels were stained with ethidium bromide [2].

a) Sterility and performance testing were carried out on one plate and one blood culture bottle from each batch in order to guarantee the prepared media's high level of quality. A blood culture bottle and a purity plate were utilized in order to check the sterility of the inoculation that was used for the biochemical tests and to determine whether or not the biochemical tests were carried out in an aseptic environment. Both of these tasks were performed in order to determine whether or not the biochemical tests were carried out in an aseptic environment. The purpose of both of these checks was to ensure that the biochemical tests were carried out in a clean setting. This was accomplished by performing both of the aforementioned checks. For the purpose of quality control, the following strains of bacteria will be utilized: E. coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, and Klebsiella pneumoniae ATCC 700603.

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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: 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

Table 2: The results on detection of *blaCTXM* family genes for *E. coli, Enterobacter spp., Klebsiella spp., and Acinetobacter spp.* shown by absolute number of bacteria and percentage.

Bacterial pathogens (n= 276)	**Detection status**	***bla*CTXM-1**		***bla*CTXM-2**		***bla*CTXM-8**		***bla*CTXM-9**		***bla*CTXM-25**	
		N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
E.Coli (n=94)	**Neg**	79	86	92	100	40	42	93	99	92	98
	Pos	13	14	0		54	58	1	1	2	2
	NT	2		2		0		0		0	
Klebsiella spp. (n=70)	**Neg**	15	22	0		0		68	99	64	98
	Pos	53	78	0		0		1	1	2	1
	NT	2		70	100	70	100	1		2	1
	Neg	11	20	0		0		61	99	0	

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Enterobacter spp. (n=63)	**Pos**	47	80	0		0		1	1	0	
	NT	5		63		63		1		63	
Acinetobacter spp. (n=49)	**Neg**	45	98	47	100	47	100	47	100	47	100
	Pos	2	2	0		0		0		0	
	NT	2		2		2		2		2	

Table 3: The results on detection of blaOXA family genes for E. coli, Enterobacter spp., Klebsiella spp., and Acinetobacter spp. shown by absolute number of bacteria and percentage

Bacterial pathogens (n= 276)	**Detection status**	***bla*$_{OXA}$**		***bla*OXA 23**		***bla*OXA 24**		***bla*OXA 48**		***bla*OXA51**		***bla*OXA 58**	
		N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
E.Coli (n=94)	**Neg**	59	63	0		0		93	99	0		0	
	Pos	45	37	0		0		1	1	0		0	
	NT	0		94		94		0		94		94	
Klebsiella spp. (n=70)	**Neg**	29	44	0		0		63	90	0		0	
	Pos	39	56	0		0		7	10	0		0	
	NT	2		70		70		0		70		70	
Enterobacter spp. (n=63)	**Neg**	27	44	0		0		42	66	0		0	
	Pos	36	56	0		0		21	44	0		0	
	NT	0		63		63		0		63		63	
Acinetobacter spp. (n=49)	**Neg**	0		30	64	43	97	0		19	42	42	96
	Pos	0		17	36	3	3	0		25	58	2	4
	NT	49		2		3		49		5		5	

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Table 4: The results on detection of blaIMP, blaVIM, blaKPC, blaNDM-1, blaTEM, and blaSHV genes for E. coli, Enterobacter spp., Klebsiella spp., and Acinetobacter spp. shown by absolute number of bacteria and percentage.

Bacterial pathogens (n= 276)	Detection status	*bla*$_{IMP}$		*bla*$_{KPC}$		*bla*NDM 1		*bla*$_{SHV}$		*bla*$_{TEM}$		*bla*$_{VIM}$	
		N	%	N	%	N	%	N	%	N	%	N	%
E.Coli (n=94)	Neg	93	99	94		88	94	94	100	9	10	82	99
	Pos	1	1	0		6	6	0		85	90	2	1
	NT	0		0		0		0		0		10	
Klebsiella spp. (n=70)	Neg	10	100	66	96	46	78	43	78	25	37	10	100
	Pos	0	0	2	4	21	22	18	22	44	63	0	
	NT	60		2		2		5		1		60	
Enterobacter spp. (n=63)	Neg	9	100	25	40	50	80	42	66	28	46	10	100
	Pos	0		38	60	13	20	21	44	35	54	0	
	NT	54		0		0		0		1		53	
Acinetobacter spp. (n=49)	Neg	0		0		33	80	44	97	34	78	0	
	Pos	0		0		11	20	2	3	10	22	0	
	NT	49		49		5		3		5		49	

Table 5: The association between presence of ESBL genes, and phenotypic status of being ESBL positivity, MDR, or non-susceptible to the third or fourth generation cephalosporins.

ESBL genes	ESBL	non-ESBL	OR (95% CI)[(c)]	p-value[(d)]
*bla*OXA[(a)]				
OXA-neg	409	461	1.4 (1.2-1.6)	0.009
OXA-pos	675	601		
*bla*CTXM[(b)]				
CTXM-neg	116	664	14.7 (11.4-	<0.001

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			18.8)	
CTXM-pos	969	408		
bla$_{TEM}$				
TEM-neg	264	519	3.6 (2.8-3.8)	<0.001
TEM-pos	817	549		
ESBL genes	**MDR**	**non-MDR**	**OR (95% CI)[c]**	**p-value[d]**
*bla*OXA[a]				
OXA-neg	752	115	2.4 (1.6- 3.0)	<0.001
OXA-pos	1196	82		
*bla*CTXM[b]				
CTXM-neg	625	145	5.90 (4.1- 8.8)	<0.001
CTXM-pos	1312	52		
bla$_{TEM}$				
TEM-neg	681	97	2.2 (1.3- 2.8)	<0.001
TEM-pos	1261	91		
ESBL genes	**Cephal. 3/4 non-susceptible[e]**	**Cephal. 3/4 susceptible**	**OR (95% CI)[c]**	**p-value[d]**
*bla*OXA[a]				
OXA-neg	562	22	1.4 (0.9- 3.1)	0.32
OXA-pos	638	18		
*bla*CTXM[b]				
CTXM-neg	266	42	29.4 (12.1-91.2)	<0.001
CTXM-pos	938	7		
bla$_{TEM}$				
TEM-neg	256	17	1.7 (1.9- 3.8)	0.21
TEM-pos	949	31		

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.

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Isolates of E. coli that were tested for specific AMR genes came back positive for blaTEM in a rate of 90%, blaCTXM-8 in a rate of 58%, and blaOXA in a rate of 37%. 56% of the Enterobacter species tested positive for the blaOXA gene, 60% of the Enterobacter species tested positive for the blaKPC gene, 80% of the Enterobacter species tested positive for the blaCTXM-1 gene, and 54% of the Enterobacter species tested positive for the blaTEM gene. 78% of Klebsiella spp. isolates tested positive for blaCTXM-1, 56% of Klebsiella spp. isolates tested positive for blaOXA, 4% of Klebsiella spp. isolates tested positive for blaKPC, and 63% of Klebsiella spp. isolates tested positive for blaTEM. In a manner analogous, 58% of Acinetobacter spp. were found to be positive for blaOXA51, 36% were found to be positive for blaOXA23, and 20% were found to be positive for blaNDM-1. 59% of the Acinetobacter spp. that were isolated in this study could be Acinetobacter baumannii species because the blaOXA51 gene has been described to occur consistently and specifically in Acinetobacter baumannii species [1-8]. The tables have been updated to include a summary of the status of E. coli, Klebsiella spp., Enterobacter spp., and Acinetobacter spp. regarding the presence of blaCTXM family genes, blaOXA family genes, and blaIMP, blaVIM, blaKPC, blaNDM-1, blaTEM, and blaSHV.

The detection of each bla_{OXA}, $bla_{\text{CTX-M}}$, and bla_{TEM} ESBL genes was significantly associated withan ESBL phenotypes. The strength of association varied by the specific ESBL genes, with the odds ratio (OR) of 1.4 (95% CI: 1.2-1.6, $p<0.05$) for bla_{OXA}, 3.6 (95% CI: 2.8-3.8, $p<0.001$) for bla_{TEM}, and 14.7 (95% CI: 11.4-18.8, $p<0.001$) for bla_{CTXM} in the Fisher's test.

Discussion:

In bacteria belonging to the family Enterobacteriaceae, such as E. coli, Enterobacter spp., and Klebsiella spp., the blaOXA, blaTEM, blaCTXM-1, and blaCTXM-8 genes were the ones that were found to be resistant to antibiotics the most frequently. The finding was in line with that of another study [6], even though the prevalence of ESBL genes was found to be lower in that other study than it was in this one. In a study that was done on clinical isolates of E. coli, the blaCTXM gene was found in 24% (23/93) of the isolates, and the blaTEM gene was found in 8% (7/93) of the isolates [7]. In Klebsiella pneumoniae, the ESBL genes with the highest frequency were found to be blaCTXM-1 (51%, 73/142) and blaSHV (27%, 38/142), according to a study that was conducted in Bangladesh [8]. According to the findings of my study [9,10], the AMR genes blaOXA, blaTEM, blaCTXM, and blaSHV were found to be prevalent in the Enterobacteriacea bacterial isolates found in Pakistan and India. According to the findings of other studies that were reported from , India, and Bangladesh [30,31,32], the Acinetobacter species genes blaOXA-51, blaOXA-23, and blaNDM-1 were the ones that were found to be the most frequently.

In particular, when 44 A. baumannii were isolated from different clinical specimens at a tertiary hospital in , all of the isolates tested positive for the blaOXA-23 gene, and 14% of

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the isolates tested positive for the blaNDM-1 gene [11]. Similarly, a study that was carried out on clinical isolates of 75 A. baumannii obtained from five centres located all over India found that 97% of the isolates tested positive for the blaOXA-23 gene, while only 17% of the isolates tested positive for the blaNDM-1 gene [32]. While another study on A. baumannii strains isolated from tracheal aspirates reported that 77% of isolates were positive for the blaOXA-23 gene and 18% (4/22) of isolates were positive for the blaOXA-58 gene [12], this study was limited to A. baumannii strains isolated. When ESBLs with reduced susceptibility to ESBL inhibitors coexist in the isolates, the phenotypic confirmatory tests used for the detection of ESBL positivity are labour intensive and frequently less sensitive [13]. There are automated systems available, but the associated costs of installation, operation, and maintenance are frequently a barrier in settings with limited resources. Alternately, polymerase chain reaction (PCR) can detect particular AMR genes with a high degree of sensitivity [15-17]. Additionally, PCR assays can be multiplexed, which cuts down on the amount of time needed for the turnaround. Before determining whether or not genetic tests like PCR are useful in clinical settings, researchers need a better understanding of the association between the phenotypic and genotypic determinants of AMR. In order to fill this gap in knowledge, the present study conducted this research with the objective of determining whether or not there is a correlation between the presence of the blaCTXM, blaTEM, and blaOXA genes identified by PCR and the presence of ESBL positivity or MDR as identified phenotypically by the disc diffusion method.

The presence of the blaCTXM gene was found to have a significant correlation with the presence of ESBLs, multidrug resistance, and resistance to cephalosporins of the third and fourth generations. The presence of blaTEM was found to have a significant correlation with the presence of ESBLs as well as MDR. And finally, the presence of blaOXA was found to be associated with ESBL as well as the MDR phenotype. According to the findings, the presence of the blaTEM, blaCTXM, or blaOXA gene in bacterial isolates appeared to be an important index for determining the MDR and ESBL phenotype.

According to the findings of this investigation, the presence of ESBL was linked to the presence of blaCTXM, blaOXA, and blaTEM. In spite of this, a sizeable proportion of the isolates that were positive for each ESBL gene turned out to be false positives in the phenotypic confirmatory test. 48 percent of GNB positive for blaOXA had a phenotype that was negative for ESBLs. This is because the Ambler class D oxacillinase that is encoded by blaOXA is only weakly inhibited by clavulanate, which is why the ESBL test produces a negative result. In a similar vein, ESBL was found to be absent in 40% of blaTEM positive GNB isolates and 30% of blaCTXM positive GNB samples. It is possible that this is due to the fact that the blaTEM gene detected by PCR encoded for a narrow spectrum TEM beta-lactamase, causing phenotypic and PCR tests to produce contradictory results. In addition, other beta-lactamases that are only partially inhibited

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by clavulanate or are not inhibited at all by it may have coexisted in the GNB isolates, which would have resulted in the detection of ESBL being masked. Additionally, Enterobacter spp. and Acinetobacter spp., which together constituted 41% of all GNB that were tested, are consistently associated with hyper-production of chromosomally encoded cephalosporinase (AmpC), which is only weakly inhibited by clavulanate [14]. Additionally, the blaAmpC gene is increasingly being found as a plasmid-encoded gene in other GNB, such as E. coli and Klebsiella spp. [36]. It's possible that the presence of both ESBL genes and blaAmpC in the bacterial isolates led to the observed discrepancy between the genetic and phenotypic confirmatory test results for ESBL positivity; this is something that needs further investigation. In this study, the researchers observed a lower level of phenotypic diversity in AMR patterns among the isolates of each bacterial genus that originated from the emergency room (ER) and outpatient departments (OPD) of the hospital, in comparison to the isolates that originated from the hospital's inpatient departments. This difference in the AMR pattern may be attributable to the fact that outpatients and inpatients use antimicrobials differently. In contrast to the outpatients, the hospital's inpatients may have been subjected to a greater variety of antimicrobials during their stays there.

Due to the lack of clinical-demographic information, such as prior exposure to hospital and/or health care institution, invasive medical devices, underlying comorbidities, and prior administration of antimicrobials, it was not possible to rule out the possibility that the isolates that originated from a subset of patients in the emergency room or outpatient department had been acquired from other hospitals.

Conclusion:

The findings of this study indicated that there was a high likelihood for GNB to be MDR and ESBL positive whenever any of the blaTEM, blaCTXM, or blaOXA genes were identified. In addition, the identification of the blaCTXM genes provided strong evidence that the GNB were resistant to the beta-lactam antimicrobials with an extended spectrum of activity.

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