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Original Research Article

Identification of Several β -Lactamases and Their Simultaneous Presence in Gram-Negative Bacteria Isolated from Clinical Specimens at a Tertiary Care Centre

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Abstract:

Background: Globally, the number of infections caused by Gram-negative bacteria is rising. In Gram negative bacteria (GNB), the extended spectrum β-lactamases (ESBLs), AmpC β-lactamases, and metallo β-lactamases (MBLs) have been identified as a source of antibiotic resistance. GNB that produces β-lactamase poses a serious diagnostic and treatment problem in the treatment of infection. Thus, the goal of the current study is to identify the antibiogram of isolates of Gram-negative bacteria, identify distinct β-lactamases and their co-existence, and assist clinicians in initiating the right antibiotic therapy for illness management.

Methods: Following the recommendations set forth by the Clinical and Laboratory Standards Institute (CLSI), a total of 150 Gram negative clinical isolates were identified, and tests for antibiotic susceptibility were conducted on them. In accordance with CLSI recommendations, the combined disk diffusion method was utilized to detect ESBL. The phenyl boronic acid test was used to identify AmpC β-lactamase. The EDTA disc potentiation test was used to identify MBL.

Result: Of the 150 Gram-negative bacteria that were examined, 26 (17.34%) produced just ESBL, and 50 (33.34%) produced only AmpC. In 16 isolates (10.67%), both ESBL and AmpC coexisted, while in 16 isolates (10.67%), AmpC and MBL co-occurred.

Conclusion: For the purpose of managing infections effectively, further testing should be conducted in addition to normal antibiotic sensitivity testing to identify "hidden" resistance mechanisms.

Keywords: Antibiogram, β-lactamases, extended spectrum β-lactamases (ESBLs), AmpCβ-lactamases, Metalloβ-lactamases (MBLs), Co-existence.

Introduction

Gram-negative bacteria are a therapeutic challenge in both community and hospital settings due to their multi-agent resistance. [1,2] Drug-resistant bacteria have been developed by hospitals and community settings using more β lactam antibiotics, which has raised morbidity, death, and health care costs. Among the several resistance mechanisms, the most prevalent and significant one is the β-lactamase-mediated cleavage of the β-lactam ring. [3]

β-lactamases, namely AmpC β-lactamases, metallo β-lactamases (MBLs), and extended spectrum β-lactamases (ESBLs), are the primary source of β-lactam resistance in Gram-negative organisms. [4] Enzymes known as ESBLs are capable of hydrolyzing a broad range of penicillins and cephalosporins, including monobactams and third-generation cephalosporins. Nevertheless, cephamycins, beta lactam plus beta lactamase inhibitor combinations, and carbapenems are effective against the bacteria that produce ESBLs. [5] Furthermore, they frequently show signs of resistance to other medication classes, including fluoroquinolones, tetracycline, aminoglycosides, and co-trimoxazole. [6,7]

The same bacteria' resistance to cephamycins and combinations of beta lactam and beta lactamase inhibitors is caused by the presence of AmpC, which restricts the available therapy options. [5,6] The ability of metallo-lactamases (MBLs) to hydrolyze almost all β-lactam drugs, including carbapenems, has made MBL-mediated resistance one of the most feared resistance mechanisms in recent times. The limited therapy options stem from its spread on highly mobile gene elements in nosocomial infections. [6–8] The efficacy of beta lac-

tam-beta lactamase inhibitor combos is reduced when ESBLs and AmpC beta lactamases are present in the same isolate, whereas MBLs and AmpC beta lactamases provide resistance to carbapenems. These enzymes are frequently expressed within the same isolate. [9,10]

Gram-negative organisms that produce β-lactamase provide a serious diagnostic and treatment challenge in the treatment of infection. This highlights how important it is to identify isolates that also produce β-lactamases in order to minimize nosocomial epidemics and therapeutic failures, shorten hospital stays, lower healthcare costs, and create an efficient antibiotic policy. Therefore, the goal of the current investigation was to identify metallo beta lactamase, AmpC beta lactamase, and ESBL in gram-negative bacteria. [11,4,12]

Material and Methods

Present study was done at Department of Microbiology, Radha Devi Jageshwari Memorial Medical College & Hospital, Turki, Muzaffarpur, and Bihar. during May 2023 to October 2023. 150 Gram-negative bacilli isolates from a variety of clinical samples that patients at the RDJMMCH, Turki, Muzaffarpur, Bihar which provided to the Microbiology Laboratory were the subject of the study.

The media used for processing these samples included blood agar, chocolate agar, and MacConkey agar. The samples were cultured under aerobic conditions at 37°C. The identification of the organisms followed accepted conventions. [13]

Based on the Kirby-Bauer disc diffusion method on Muller-Hinton agar, isolates were tested for antibiotic susceptibility using the Clinical and Laboratory Standards Institute (CLSI, 2018) recommendations. [7] Ampicillin (AMP), Ampicillin/sulbactam (A/S), Cefuroxime (CXM), Ceftazidime (CAZ), Co-trimoxazole (COT), Gentamicin (G), Chloramphenicol (c), Ciprofloxacin (CIP), Imipenem (IMP), Meropenem (MRP), Piperacillin-tazobactam (PT), Amikacin (AK), Cefoxitin (cx), Cefepime (CPM), and Aztreonam (AT) were the antibiotics used in the susceptibility testing. [14]

Results

Table 1: Organisms isolated from clinical samples

Organisms	No. of samples	Percentage
Escherichia coli	56	37.34%
Klebsiella spp.	40	26.67%
Pseudomonas aeruginosa	24	16.00%
Acinetobacter spp.	16	10.67%
Proteus spp.	8	5.34%
Citrobacter spp.	4	2.60%
Enterobacter spp.	2	1.34%
Total	150	100%

Table 2: Organism-wise distribution of different beta lactamases and their co-production

Organisms	Pure ESBL N (%)	Pure AmpC N (%)	ESBL + AmpC N (%)	AmpC+ MBL N (%)	Non beta lactamase Producer N (%)	Total N (%)
Escherichia coli	18 (12.00)	14 (9.34)	6 (4.00)	6 (4.00)	12 (8.00)	56 (37.34)
Klebsiella spp.	4 (2.60)	12 (8.00)	8 (5.34)	4 (2.60)	12 (8.00)	40 (26.67)
Pseudomonas aeruginosa	2 (1.34)	16 (10.67)	0	2 (1.34)	4 (2.60)	24 (16.00)
Acinetobacter spp.	2 (1.34)	6 (4.00)	2 (1.34)	2 (1.34)	4 (2.60)	16 (10.67)
Proteus spp.	0	0	0	0	8 (5.34)	8 (5.34)
Citrobacter spp.	0	0	0	2 (1.34)	2 (1.34)	4 (2.60)
Enterobacter spp.	0	2 (1.34)	0	0	0	2 (1.34)
	26 (17.34)	50 (33.34)	16(10.67)	16 (10.67)	42 (28.00)	150 ((100)

Of the 150 Gram-negative bacteria that were examined, 26 (17.34%) produced just ESBL, and 50 (33.34%) produced only AmpC. In 16 isolates (10.67%), ESBL and AmpC co-occurred, and in 16 isolates (10.67%), AmpC and MBL co-occurred.

Table 3: Distribution of different beta lactamases and their co-production

	No. of samples	Percentage
Pure ESBL	26	17.34%
Pure AmpC	50	33.34%
ESBL + AmpC	16	10.67%
AmpC + MBL	16	10.67%
No beta lactamase	42	28.0%

Table 4: Antibiotic sensitivity pattern of β-lactamase producing gram negative bacteria

	β-lactamase producers						
Antibiotics tested	**Escherichia coli N-48(%)**	**Klebsiella spp. N-28(%)**	**Acinetobacter spp. N-10(%)**	**Proteus spp N-0**	**Citrobacter spp N-2(%)**	**Enterobacter spp N-2(%)**	**Pseudomonas spp. N-20(%)**
Ampicillin	0	0	0	-	0	0	-
Gentamicin	28(58.33)	10(35.71)	6(60.00)	-	0	1(50.00)	6(30.00)
Amikacin	36(75.00)	15(53.57)	7(70.00)	-	1(50.00)	1(50.00)	10(50.00)
Ampicillin/sulbactam	6(12.50)	4(14.28)	4(40.00)	-	0	0	-
Cefuroxime	7(14.58)	5(17.85)	2(20.00)	-	0	0	-
Cefoxitin	10(20.83)	7(25.00)	2(20.00)	-	0	0	-
Cefepime	10(20.83)	10(35.71)	6(60.00)	-	1(50)	1(50.00)	6(30.00)
Ciprofloxacin	24(50.00)	15(53.57)	4(40.00)	-	2(100)	0	10(50.00)
Imipenem	42(87.50)	22(78.57)	8(80.00)	-	1(50.00)	2(100)	18(90.00)
Meropenem	16(33.34)	18(64.28)	4(40.00)	-	1(50.00)	1(50.00)	16(80.00)
Co- trimoxazole	16(33.34)	7(25.00)	0	-	0	0	-
Aztreonam	10(20.83)	7(25.00)	2(20.00)	-	1(50.00)	1(50.00)	13(65.00)
Ceftazidime	10(20.83)	8(28.57)	5(50.00)		1(50.00)	1(50.00)	8(40.00)
Chloramphenicol	36(75.00)	17(60.71)	4(40.00)		2(100)	2(100)	-
Piperacillin- tazobactam	-	-	-		-	-	9(45.00)

Table 5: Antibiotic sensitivity pattern of non-β-lactamase producer

	Non-β-lactamase producers						
Antibiotics tested	**Escherichia coli N-8(%)**	**Klebsiella spp. N-12(%)**	**Acinetobacter spp N-6(%)**	**Proteus spp N-8(%)**	**Citrobacter spp N-2(%)**	**Enterobacter spp N-0**	**Pseudomonas spp N-4(%)**
Ampicillin	0	0	0	0	0	0	-
Gentamicin	7 (87.50)	8 (66.67)	4 (66.67)	4 (50.00)	2 (100)	0	3 (75.00)
Amikacin	6 (75.00)	8 (66.67)	4 (66.67)	4 (50.00)	2 (100)	0	3 (75.00)
Ampicillin/sulbactam	0	2 (16.67)	2 (33.34)	4 (50.00)	2 (100)	0	-
Cefuroxime	4 (50.00)	6 (50.00)	4 (66.67)	4 (50.00)	0	0	-
Cefoxitin	5 (62.50)	6 (50.00)	5 (83.34)	7 (87.50)	2 (100)	0	-
Cefepime	7 (87.50)	9 (75.00)	5 (83.34)	7 (87.50)	2 (100)	0	2 (50.00)
Ciprofloxacin	6 (75.00)	9 (75.00)	4 (66.67)	6 (75.00)	2 (100)	0	2 (50.00)
Imipenem	8 (100)	12 (100)	6 (100)	8 (100)	2 (100)	0	4 (100)
Meropenem	8 (100)	9 (75.00)	5 (83.34)	7 (87.50)	2 (1000	0	4 (100)
Co- trimoxazole	6 (75.00)	7 (58.33)	4 (66.67)	5 (62.50)	1 (50.00)	0	-
Aztreonam	7 (87.50)	10 (83.34)	4 (66.67)	7 (87.50)	2 (100)	0	4 (100)
Ceftazidime	4 (50.00)	9 (75.00)	2 (33.34)	6 (75.00)	2 (100)	0	2 (50.00)
Chloramphenicol	6 (75.00)	9 (75.000	5 (83.34)	7 (87.50)	2 (100)	0	-
Piperacillin- tazobactam	-	-	-	-	-	-	3 (75.00)

Discussion

Gram-negative bacteria (GNB) have become resistant to antibiotics due to the β-lactamases, which include metallo-β-lactamases, Amp-C-β-lactamases, and extended-spectrum β-lactamases. This phenomenon has been observed globally. The genes for all three of these enzymes are frequently carried on plasmids, allowing for the quick transmission of germs. These enzymes are frequently co-expressed in the same isolate. [9]

The emergence of Gram-negative microbes that produce β-lactamase poses a significant challenge to infection management in terms of both diagnosis and treatment. 50 (33.34%) of the 150 gram negative bacteria we examined in our investigation were pure AmpC producers, while 26 (17.34%) were pure ESBL producers. AmpC and ESBL co-occurred in 16 isolates (10.67%). MBL and AmpC co-occurred in 16 isolates (10.67%). The current investigation revealed that certain Gram negative bacteria isolated from our hospital have a high in-

cidence of AmpC beta lactamases. Risk factors for colonization or infection with these organisms likely include lengthy hospital stays, admissions to intensive care units, intravenous and urine catheterization, and antibiotic exposure, notably extended spectrum cephalosporins. [12]

A 2013 study conducted in Uttarakhand revealed that out of the 184 gram-negative bacteria examined, 30 (16.3%) were pure ESBL producers, 26 (14.1%) were pure AmpC producers, and 60 (32.6%) were pure MBL producers. Additionally, 42 (22.8%) and 16 (8.6%) isolates showed co-occurrences of ESBL and AmpC. Of the 251 isolates examined in the 2007–2008 study conducted in Uttar Pradesh, 138 (54.98%) produced ESBL, 49 (19.52%) produced AmpC, and 45 (17.93%) produced MBL.[7] A study conducted in Hyderabad between November 2010 and October 2011 found that out of 200 Gram negative isolates, 50 (or 25%) had pure ESBL and 35 (17.5%) had pure AmpC. In 38(19%) isolates, ESBL and AmpC coexisted. MBL and AmpC were found together in a single isolate. [4]

Comparing β-lactamase producers to non-producers, we found that antibiotic resistance was higher in our study. These findings are consistent with research conducted in Pondicherry in 2011 and Hyderabad from November 2010 to October 2011. This might be because the plasmids containing these enzymes also contain genes that confer resistance to other drugs. [15]

The presence of many beta lactamases in gram-negative bacteria restricts treatment alternatives and presents a diagnostic difficulty for microbiologists. The efficacy of beta-lactam–beta-lactamase inhibitor combinations is decreased when ESBLs and AmpC beta-lactamases are present in a single strain, whereas carbapenem resistance is conferred by MBLs and AmpC beta-lactamases. [5,9] The identification of beta lactamases in GNB is crucial for epidemiological research, infection control, and the effective treatment of infections with the right drugs.

Conclusion

Special tests should be conducted in addition to standard antibiotic sensitivity testing to identify these "hidden" resistance mechanisms, as referrals for therapy based solely on antibiotic susceptibility reports without β-lactamase testing may result in serious therapeutic failures. In a typical diagnostic laboratory, phenotypic tests with a variety of substrates and inhibitors are a straightforward and straightforward method for detecting beta lactamases.

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