Prevalence Of Drug Resistance In Gram Negative Isolates In A Tertiary Care Hospital Of North Zone, India

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Abstract: Background: The rapid emergence of antibiotic resistance in Gram negative bacteria is becoming a serious threat to management of infectious diseases. Patients with antibiotic resistant gram negative infections are going to have increased morbidity and mortality. Beta-lactamases are a family of enzymes involved in bacterial resistance to antibiotics. This study is planned to see antimicrobial susceptibility pattern of Gram negative isolates along with prevalence of ESBL, AmpC β-lactamase and Carbapenemase producers. Methods: A prospective study conducted over a period of two months in Microbiology Department. All samples (sputum, endotracheal secretions, bronchoalveolar lavage, urine, pus, blood, body fluids etc.) were included. Results: A total of 612 Gram negative isolates were studied. Respiratory, pus and blood samples: Klebsiella, Proteus was minimally sensitive to drugs like cephalosporins, aminoglycosides, quionolones and monobactams. E.coli (7.9%), Acinetobacter (63.8%), Klebsiella (51.7%) are confirmed ESBL, carbapenemase and AmpC β lactamases producers respectively. Urine samples: were least sensitive to drugs like beta lactams, beta lactamase inhibitors, cephalosporins, aminiglycosides. Enterobacter (83.3%), Pseudomonas (77.8%), Klebsiella (21.9%) are confirmed ESBL, carbapenemases and AmpC β lactamases producers respectively. Interpretation & Conclusion: The increase in prevalence of β lactamase producing isolates is indicating increasing trend of isolates acquiring resistance mechanisms and narrowing down treatment options available for empiric therapy against infections.[Mahajan A Natl J Integr Res Med, 2020; 11(5):11-16]

Key Words: Antibiotic resistance, Beta-lactamases, Gram negative isolates

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Introduction: Gram negative bacteria are among the most important human pathogens accounting for majority of bacterial isolates from clinical samples. The different Gram negative organisms include members of family Enterobacteriaceae, Acinetobacter spp., Pseudomonas aeruginosa and various other organisms[1]. They cause a wide range of infections like pneumonia, blood stream infections, wound or surgical site infections, meningitis etc. The rapid emergence of antibiotic resistance among these pathogens is becoming a serious threat to the management of infectious diseases[2].

There are various mechanisms such as enzymatic inactivation of antibiotics, altered target sites, decreased porin permeability and active efflux pumps, which are responsible for production of multidrug resistant organisms (MDROs). MDROs are resistant to one or more classes of antimicrobial agents such as β lactam including penicillins, cephalosporins, monobactams, carbapenems, floroquinolones and aminiglycosides. The prevention and control of MDROs should be a national priority[3].

Extended-spectrum β lactamases (ESBLs) are a rapidly evolving group of β lactamases which share the ability to hydrolyze third-generation cephalosporins and aztreonam[4]. Currently, a majority of the clinical laboratories test for production of ESBLs. However, the testing of clinical isolates for production of plasmid mediated AmpC β lactamases is usually ignored. AmpC β lactamases are also associated with multiple antimicrobial resistances, which limit the therapeutic regimens[5,6]. Due to increase in incidence of MDROs, carbapenems are considered the last resort to combat infections. They are presently considered as the most potent agents for treatment of multidrug resistant Gram-negative infections due to stability of these agents against majority of β lactamases and their high rate of permeation through bacterial outer membranes.

However, resistance to carbapenems is also being reported[7]. Some of the isolates show more than one resistance mechanism by synthesizing a combination of 2 or 3 enzymes. This increase in incidence of multidrug resistance has led to the study of various resistance mechanisms along with prevalence of Gram negative bacteria.

Aims And Objectives: To study the antimicrobial susceptibility pattern of the Gram negative isolates and to categorize them into MDR, XDR, and PDR. To study the prevalence of ESBL, AmpC

β-lactamase and Carbapenemase producers among the isolates.

Material and Methods: This was a prospective study which was conducted over a period of two months in the Department of Microbiology of a tertiary care hospital. Institutional Ethical committee (IEC) approval was taken before start of study. As the study involves only collection of laboratory data and there is no intervention, informed consent waiver was requested from IEC.

Screening tests were done in Microbiology Department, which helped to differentiate between ESBL/AmpC β-lactamases/Carbapene mase producing organisms.

Statistical Analysis: Data obtained from the study was entered in Microsoft Excel sheet. Descriptive statistics was computed by using statistical software SPSS 20.0.

Results : The study was conducted for a period of 2 months in department of microbiology.

- Number of total samples (urine) received in:
- May, 2017: 1161
- June, 2017: 1345
- Number of total samples (urine) positive for Gram negative isolates (May, June, 2017): 151
- Number of total samples (Respiratory, Pus and Blood) received in:
- May, 2017: 2121
- June, 2017: 1884

Source Of Data: All the samples (sputum, endotracheal secretions, bronchoalveolar lavage, urine, pus, blood and body fluids etc.) coming to microbiology department were included in the study. On the basis of antimicrobial susceptibility testing, Gram negative isolates were characterized into:

Multi Drug Resistance (MDR): Non susceptible to ≥ 1 agent in ≥ 3 antimicrobial categories.

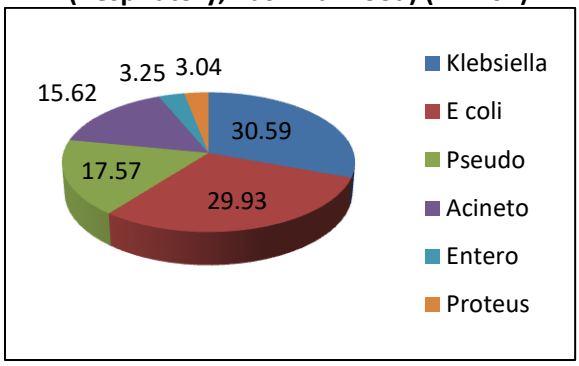
Extensively Drug Resistance (XDR): non susceptible to ≥ 1 agent in all but ≤ 2 antimicrobial categories.

Pan Drug Resistance (PDR): Non susceptible to all antimicrobial agents.

- Number of total samples (Respiratory, Pus and Blood) positive for Gram negative isolates (May, June, 2017): 461
- Total no. of Gram negative isolates (May, June, 2017): 151+461 = 612

Among the Gram negative isolates, Klebsiella spp. (30.59%), E.coli (29.93%) were the most common followed by Pseudomonas aeruginosa (17.57%) Acinetobacter spp. (15.62%) (Fig1).

Figure 1: Distribution Of Gram Negative Isolates (Respiratory, Pus And Blood) (N= 461)



The antimicrobial susceptibility pattern of Gram negative isolates in Respiratory, Pus and Blood samples is depicted in Table 1.

Table 1: Antimicrobial Susceptibility Pattern Of Gram Negative Isolates (Respiratory, Pus And Blood Samples)

Antibiotics	Klebsiella	E coli	Pseudomonas	Acinetobacter	Enterobacter	Proteus
	N=141	N=138	N=81	N=72	N=15	N=14
Amoxicillin+Clavulanic acid	18 (12.77)	50 (36.23)	-	-	-	-
Piperacillin+Tazobactam	41 (29.08)	72 (52.17)	7 (8.64)	7 (9.72)	6 (40.0)	7 (50.0)
Cefoperazone+ Sulbactam	45(31.91)	81 (58.70)	44 (54.32)	9(12.50)	5 (33.33)	8 (57.14)
Cefuroxime	7 (4.96)	6 (4.35)	-	-	-	-
Ceftriaxone	5 (3.55)	2 (1.45)	-	-	-	-
Ceftazidime	5 (3.55)	6 (4.35)	41 (50.62)	5 (6.94)	6 (40.00)	4 (28.57)
Cefepime	11 (7.80)	12 (8.70)	36 (44.44)	4 (5.56)	7 (46.67)	6 (42.86)
Dorepenem	-	-	44 (54.32)	1 (1.39)	3 (20.00)	0 (0.00)
Ertapenem	52 (36.88)	90 (65.22)	29 (35.80)	-	-	-

Imipenem	51 (36.17)	90 (65.22)	29 (35.80)	2 (2.78)	3 (20.00)	5 (35.71)
Meropenem	53 (37.59)	96 (69.57)	37 (45.68)	4 (5.56)	8 (53.33)	8 (57.14)
Amikacin	52 (36.88)	124(89.86)	45 (55.56)	7 (9.72)	8 (53.33)	9 (64.29)
Gentamicin	39 (27.66)	87 (63.04)	42 (51.85)	5 (6.94)	7 (46.67)	8 (57.14)
Ciprofloxacin	0 (0.00)	5 (3.62)	37 (45.68)	3 (4.17)	7 (46.67)	7 (50.00)
Levofloxacin	32 (22.70)	3 (2.17)	20 (24.69)	2 (2.78)	4 (26.67)	5 (35.71)
Minocycline	-	-	3 (3.7)	20 (27.8)	1 (6.7)	0 (0.00)
Tigecycline	94 (66.67)	136(98.55)	4 (4.94)	67 (93.06)	14 (93.33)	4 (28.57)
Colistin	126(89.4)	114 (82.6)	0 (0.0)	63 (87.5)	9 (60.0)	IR*
Cotrimoxazole	32 (22.70)	49 (35.51)	7 (8.64)	11 (15.28)	11 (73.33)	7 (50.00)

IR*= Intrinsically Resistant

Klebsiella spp, E. coli have shown maximum sensitivity towards colistin 89.4%, 82.6% respectively while the susceptibility is only 3.55% and 1.45% respectively to ceftriaxone. The Gram negative isolates were also categorized into MDR/XDR/PDR (Table 2).

Table 2: Categorization Of Gram Negative Isolates (Respiratory, Pus, Blood) Into MDR/XDR/PDR

Organism (N)	MDR N (%)	XDR N (%)	PDR N (%)
Klebsiella (141)	87 (61.7)	47 (54.0)	2 (4.2)
E coli (138)	120(86.9)	2 (1.6)	0
Pseudomonas (81)	36 (44.4)	1(2.7)	0
Acinetobacter (72)	61(84.7)	11(18.0)	0
Enterobacter (15)	9 (60)	4(44.4)	0
Proteus (14)	9(64.2)	3 (33.3)	0

Probable ESBL, AmpC and Carbapenemase producers were further subjected to confirmatory tests and after which they were labelled as confirmed ESBL, confirmed AmpC and confirmed Carbapenemases producers (Table 3).

Table 3: β Lactamase Production Among Gram Negative Isolates (Respiratory, Pus And Blood)

	ESBL Producers (%)		Carbapenemases Producers (%)		AmpC Producers (%)	
Organism	Probable	Confirmed	Probable	Confirmed	Probable	Confirmed
Klebsiella	95.03	26.9	63.8	56	82.9	51.7
E coli	98.55	57.9	26.8	15.9	77.5	16.6
Pseudomonas	40.74	6.1	64.1	44.4	41.9	27.1
Acinetobacter	93.05	6.9	97.2	63.8	79.1	40.2
Enterobacter	60	6.6	66.6	33.3	33.3	6.6
Proteus	71.42	35.7	64.2	28.5	57.1	21.4

Maximum ESBL producers were E.coli, highest carbapenemase producers were Aceinetobacter and AmpC production was highest in Klebsiella.

Gram negative urinary isolates (n=151): E.coli (58.27%), Klebsiella spp.(21.19%), Pseudomonas aeruginosa (11.92%) were the most common followed by Enterobacter (3.97%), Proteus spp (3.31 %) and Acinetobacter spp.(1.32%). Based on antimicrobial susceptibility pattern, E.coli was less sensitive to Amoxicillin+Clavulanic acid (2.27%), Ofloxacin (7.95%) while sensitivity was 68.2% to Polymixin B (Table 4).

Table 4: Antimicrobial Susceptibility Pattern Of Gram Negative Isolates (Urine Samples)

Antibiotics	E coli	Klebsiella	Pseudo	Entero	Proteus	Acineto
	N=88	N=32	N=18	N=6	N=5	N=2
Ampicillin	20(22.73)	-	-	-	3(60.0)	1(50.0)

Cephlaxin	1(1.14)	2(6.25)	-	-	1(20.0)	-
Cefuroxime	6(6.82)	5(15.63)	-	-	3(60.0)	-
Ceftazidime	5(5.68)	4(12.50)	2(11.11)	-	3(60.0)	1(50.0)
Ceftriaxone	6(6.82)	4(12.50)	-	1(16.67)	3(60.0)	1(50.0)
Cefoperazone Sulbactam	10(11.36)	1(3.13)	1(5.56)	-	1(20.0)	1(50.0)
Amoxicillin+Clavulanic acid	2(2.27)	-	-	-	3(60.0)	1(50.0)
Piperacillin+Tazobactam	38(43.18)	8(25.0)	3(16.67)	-	4(80.0)	1(50.0)
Ertapenem	59(67.05)	1(3.13)	-	3(50.0)	2(40.0)	-
Gentamicin	11(12.50)	8(25.0)	6(33.3)	2(33.3)	3(60.0)	1(50.0)
Amikacin	74(84.09)	13(40.63)	5(27.78)	2(33.3)	3(60.0)	1(50.0)
Cotrimoxazole	31(35.23)	5(15.63)	-	1(16.67)	1(20.0)	1(50.0)
Nitrofurantoin	65(73.86)	1(3.13)	-	-	-	-
Fosfomycin	88(100.0)	25(78.13)	6(33.33)	4(66.67)	4(80.0)	-
Norfloxacin	10(11.36)	8(25.0)	3(16.67)	1(16.67)	2(40.0)	-
Ofloxacin	7(7.95)	8(25.0)	2(11.11)	2(33.3)	1(20.0)	-
Polymixin B	60(68.18)	16(50.0)	8(44.44)	4(66.67)	-	2(100)

The Gram negative isolates were also categorized into MDR/XDR/PDR (Table 5)

Table 5: Categorization Of Gram Negative Isolates (Urine Samples) Into MDR/XDR/PDR

Organisms (N)	MDR N(%)	XDR N(%)	PDR N(%)
E coli (88)	81(92.0)	7(8.6)	0(0.0)
Klebsiella (32)	16(50.0)	10(62.5)	3(30.0)
Pseudomonas (18)	8(44.4)	6(75.0)	4(66.6)
Enterobacter (6)	4(66.7)	2(50.0)	0(0.0)
Proteus (5)	3(60.0)	2(66.6)	0(0.0)
Acinetobacter (2)	2(100.0)	1(50.0)	0(0.0)

Out of 151 Gram negative isolates from urine samples, Probable ESBL, probable AmpC and probable Carbapenemase producers were further subjected to confirmatory tests and were labelled as confirmed ESBL, confirmed AmpC and confirmed Carbapenemase producers (Table 6).

Table 6: β Lactamase Production Among Gram Negative Isolates (Urine Samples)

	ESBL Producer		**Carbapenemases Producers**		**AmpC Producers**	
Organism	**Probable**	**Confirmed**	**Probable**	**Confirmed**	**Probable**	**Confirmed**
E coli	92.0	48.9	89	31.8	55.7	3.6
Klebsiella	81.3	40.6	81	50.0	71.9	21.9
Pseudomonas	100	72.2	89	77.8	83.3	27.8
Enterobacter	83.3	83.3	67	33.3	83.3	16.7
Proteus	80.0	20.0	80	20.0	60.0	20.0
Acinetobacter	50.0	50.0	100	100	0	0

Discussion: This prospective study was done in the department of Microbiology over a period of two months. Out of 612 Gram negative isolates, 461 samples were from respiratory, pus and blood ; 151 were from urine samples. Amongst the respiratory, pus and blood samples, the most common isolate was Klebsiella (n=141) 30.59% followed by E.coli (n=138) 29.93%. The most common isolate from urine sample was E.coli (n=88) 58.27% followed by Klebsiella (n=32) 21.19%. E. coli is also being observed as the most common isolate followed by Klebsiella, Pseudomonas from pus and urine samples in another study[8].

The pattern of drug resistance keeps on changing frequently with use and withdrawal of drugs. So, the resistance pattern of all organisms can serve as good guide for future empiric therapy. In our study, amongst the various Gram negative isolates from respiratory, pus and blood samples, Klebsiella, one of the common pathogens was least sensitive to drugs like ceftriaxone,

ceftazidime, ciprofloxacin, cotrimoxazole, levofloxacin, Gentamicin, Ertapenem ranging from 3.55% to 36.88%. Proteus was minimally sensitive to drugs like ceftazidime, Tigecycline, Levofloxacin, Imipenem ranging from 28.57% to 35.71%. Similarly, other isolates like E.coli, Pseudomonas, Aceinetobacter, Enterobacter have also shown reduced sensitivity to different group of antibiotics (Table1).

The isolates from urine samples in our study like E. coli (most common pathogen) is least sensitive to drugs like Amoxicillin and Clavulanic acid, Norfloxacin, Cephlexin, Cefuroxime, cefoperazone Sulbactam, Gentamicin ranging from 2.27% to 12.50%. Similarly, other isolates like Klebsiella, Pseudomonas, Enerobacter, Proteus and Aceinetobacter spp. have also shown reduced sensitivity to different group of antibiotics (Table4).

The isolates from respiratory, pus and blood samples like E.coli has shown 86.9% multi drug resistant, 1.6% of it is extensively drug resistant. Pan drug resistant was seen in 4.2% of Klebsiella (Table2). E.coli being the most common isolate in urine samples in our study is 92% multi drug resistant, 8.6% of it is extensively drug resistant. Pan drug resistance was seen in 30% of Klebsiella, 66.6% of Pseudomonas (Table5).

The incidence of extended spectrum β lactamases (ESBLs), AmpC β lactamases and carbapenemases among Gram negative organisms has increased in recent years. Prescription of antibiotics to control infection is the basic treatment offered but drug resistance to multiple classes of drugs is becoming another huge problem in these pathogens.

Our study has shown that in respiratory, pus and blood samples, 57.9% of E.coli is confirmed ESBL producer, Acinetobacter is 63.8% carbapenemases producer and 51.7% of Klebsiella is AmpC β lactamases producer (Table 3).

In urine samples of our study, 83.3% of Enterobacter are confirmed ESBL producer, 77.8% of Pseudomonas is carbapenemases producer and 21.9% of Klebsiella is AmpC β lactamases producer (Table6).

The only β-lactams which were effective against ESBL and AmpC were Carbapenems, however recently the resistance due to carbapenemase production has been increasing.

The increase in prevalence of β lactamase producing isolates are indicating the increasing trend of more and more isolates acquiring the resistance mechanisms and narrowing down the treatment options available for empiric therapy against infections.

Therefore, early detection in routine laboratory, immediate infection control and antibiotic stewardship programs should be implemented in order to limit the spread of β lactamase producing organisms.

Conclusions: Antimicrobial drug resistance is emerging worldwide as a major public health problem. Selective pressure of misuse and overuse of antibiotics in the community as well as in the hospitals has resulted in the emergence and dissemination of resistant bacteria in the hospitals. In view of this emerging drug resistance the practice of routine testing along with conventional antibiogram would be useful in proper treatment of patient and will also prevent further development of drug resistance.

This study emphazises the need for a continuous surveillance in hospitals to detect resistant strains, strict guidelines for antibiotic therapy and implementation of infection control measures to reduce increasing burden of antibiotic resistance.

References:

1. Winn WC, Allen SD, Janda WM, Koneman EW, Procop Gary, Schreckenber PC et al. Mycology. In: Konmann's atlas and Textbook of Diagnostic Microbiology 6th edition. Lippincott Wilkins. 2006.
2. Singh N, Pattanaik D, Neogi DK, Jena J, Mallick B. Prevalence of ESBL in Escherichia coli isolates among icu patients in a tertiary care hospital. J Clin Diag Res. 2016;10(9): 19-22.
3. Merchant M, Karnad DR, Kanbur AA. Incidence of nosocomial pneumonia in a medical intensive care unit and general medical ward

patients in a public hospital in Bombay, India. J Hosp Infect. 1998 Jun;39(2):143-8.
4. Philippon A, Labia R, Jacoby G. Extended-spectrum beta-lactamases. Antimicrob Agents Chemother. 1989 Aug;33(8):1131-6.
5. Bradford PA, Urban C, Mariano N, Projan SJ, Rahal JJ, Bush K. Imipenem resistance in Klebsiella pneumoniae is associated with the combination of ACT-1, a plasmid-mediated AmpC beta-lactamase, and the foss of an outer membrane protein. Antimicrob Agents Chemother. 1997 Mar;41(3):563-9.
6. Rodríguez-Martínez JM, Pascual A, García I, Martínez-Martínez L. Detection of the plasmid-mediated quinolone resistance determinant qnr among clinical isolates of Klebsiella pneumoniae producing AmpC-type beta-lactamase. J Antimicrob Chemother. 2003 Oct;52(4):703-6.
7. Gupta E, Mohanty S, Sood S, Dhawan B, Das BK, Kapil A. Emerging resistance to carbapenems in a tertiary care hospital in north India. Indian J Med Res. 2006 Jul;124(1):95-8.
8. Shinu P, Bareja R, Goyal M, Singh VA, Mehrishi P, Bansal M et al. Extended-spectrum β lactamase and AmpC β lactamase production among gram-negative bacilli isolates obtained from urinary tract infections and wound infections. Indian J Clin pract. 2014;24(11):1019-26.

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