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Antibiogram of Gram Negative Bacterial Isolates From Intensive Care Unit At A Tertiary Care Hospital

KEYWORDS: Gram negative bacilli, Antibiotics, Multidrug resistance.

* Ms.Reena Rajan	Dr.A.Venkata Raghavendra Rao
Senior Lecturer in Microbiology, Penang International Dental College, Vinayaka Mission s University,Salem-636308 * corresponding author	Assistant Professor, Microbiology, V.M.K.V.Medical college, Chinnaseeragapadi, Salem-636308,

ABSTRACT *Gram negative bacterial infections continue to be one of the leading causes of morbidity and mortality in ICUs. The knowledge of antibiotic resistance pattern of these isolates facilitates effective empirical therapy, thereby preventing the emergence and spread of resistant microorganisms. This study is done to determine the antibiotic resistance pattern of Gram negative bacilli (GNB) from ICUs.*

GNB obtained from various clinical specimens were identified by standard biochemical methods & Antibiotic sensitivity testing was performed.

Out of 696 samples studied, GNB was isolated from 185 (26.58%). The predominant GNB isolated were Klebsiella spp, followed by Acinetobacter spp, E.coli, Pseudomonas spp and Proteus.spp. About 69.73% of GNB were resistant to the fluoroquinolone tested. Among the aminoglycosides, 57.84% were resistant to netilmicin, 61.08% to amikacin and 70.81% to gentamicin, and 67.00% to third generation cephalosporins. Acinetobacter spp was the most resistant isolate to the antimicrobials tested.

INTRODUCTION:

Multidrug resistance among gram negative bacilli is an emerging clinical problem in intensive care units. Nosocomial infections accounts for high morbidity and mortality rate among hospitalized patients (1). The dissemination of multidrug resistant strains of Gram negative bacilli continue to become a major therapeutic problem in most parts of the world(2). Hospital-acquired infections caused by GNB are difficult to manage, due to various resistance mechanisms these bacteria can develop. The various mechanism of drug resistance in bacteria is commonly mediated by beta-lactamases and aminoglycoside modification,decreased permeability of antibiotics through bacterial cell wall,altered target sites of antibiotics action,bacterial efflux mechanisms(3).

Among gram-negative bacteria, the resistance is mainly due to increase of Extended-Spectrum β-Lactamases (ESBLs) in *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis, Enterobacter spp.* and *Citrobacter spp.*, and Multidrug resistance(MDR) in *Pseudomonas aeruginosa,* and *Acinetobacter spp.* (3,4). This limits the choice of antibiotics used for treatment.Hence the knowledge of antibiotic resistance pattern of these isolates facilitates effective empirical therapy.

AIMS &OBJECTIVES:

1. To isolate and identify gram negative bacilli causing infections in patients admitted to medical and surgical ICUs.

2. To determine their antibiotic resistance pattern

MATERIALS AND METHODS:

This study was conducted at the department of microbiology, over a period of 6 months from November 2013 to April 2014.The specimens collected were blood , urine, endotracheal aspirate, sputum, bronchial washing, wound swab , bodyfluids etc. All the samples were inoculated on to Blood agar and Mac Conkey agar and incubated overnight at 37°C for 24 to 48 hrs.The colonies were subjected to gram staining and screened for gram negative isolates. Identification of isolates were done by the following test ;Catalase, Oxidase, Triple Sugar Iron agar , Citrate Utilization , Indole , Methyl red , Voges Proskaur , Urease (5).

Antibiotic susceptibility testing was done on Muller Hinton agar by Kirby bauer disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) recommendations (6) .The antibiotics used were Amikacin (30µg), Cefaperazone /Sulbactam (75/30µg), Piperacillin/ tazobactam (100µg), Ceftazidime (30µg), Cefotaxime (30 µg), Cefepime (30µg), Imipenem (10µg), Levofloxacin (5 µg),Netilmicin (30 µg) , Ciprofloxacin (5µg), Gentamicin (10 µg), Tigecycline (15 µg), Colistin (10 µg) and, Polymyxin B (300 units). Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as a quality control (6) The zone of inhibition was measured and interpreted as susceptible ,intermediate or resistant.

RESULTS:

The samples were collected from 501 patients of Medical ICU and 195 patients of Surgical ICU. **[Figure 1]**.Out of 696 samples studied, GNB was isolated from 185 (26.58%).

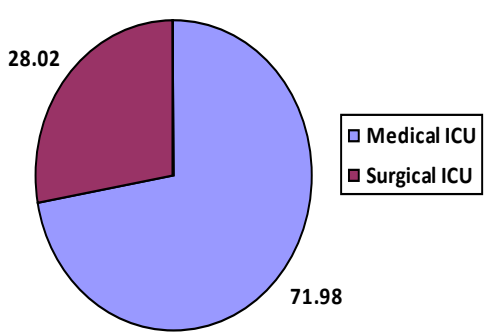

Figure1: Percentage distribution of samples from ICU

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The frequently isolated Gram-negative bacilli were *Klebsiella* spp 64 (34.59%) followed by *Acinetobacter spp* 50 (27.03%), *E.coli* 32 (17.30%), *Pseudomonas spp* 31 (16.76%), and *Proteus.spp* 8 (4.32%).

Ecoli (37.50%) the most frequent isolates in urinary tract infection followed by Klebsiella (25.00%) and Acinetobacter spp(25.00 %).

Klebsiella spp (46.77% 29/62) were the most frequent isolates from respiratory specimens .This is followed by Pseudomonas spp (25.81%) and Acinetobacter spp (20.97%) **[Table: 1]**

Table:1 Distribution of gram negative bacilli obtained from various clinical samples.

Organisms	Urine	Sputum	Endotracheal aspirate	Bronchial washing	Pus	Woundswab	Blood	Body fluid	
E.coli	24	1	2	0	0	4	0	1	32
Klebsiella spp	16	7	22	0	3	8	4	4	64
Acinetobacter Spp	16	4	8	1	3	5	5	8	50
Pseudomonas spp	6	2	13	1	3	3	2	1	31
Proteus spp	2	0	1	0	0	3	0	2	8
Total	64	14	46	2	9	23	11	16	185

About 69.73% of gram negative isolates studied were found to be resistant to the fluoroquinolone tested. Among the aminoglycosides studied, 57.84% (107/185) were resistant to netilmycin, 61.08% (113/185) were resistant to amikacin and 70.81% (131/185) were resistant to gentamicin. About 67.00% isolates were resistant to third generation cephalosporins. [**Figure2]**

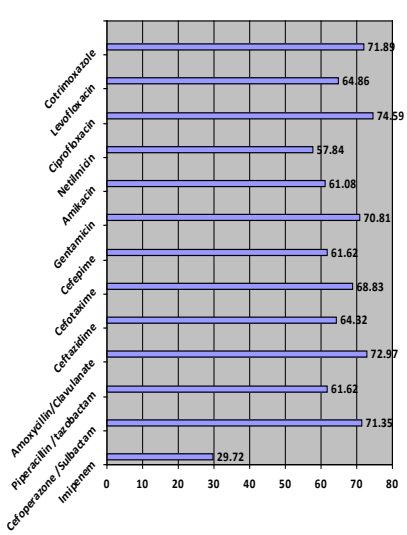

Figure 2: Overall resistance pattern of Gram negative bacilli

Among the Gram negative bacilli studied, Acinetobacter was the most resistant isolate to the antimicrobials tested [Table 2]. The percentage resistance of Acinetobacter spp to Imipenem was found to 52.00.

Table:2 Resistance pattern of various gram negative bacilli

Antibiotics	Klebsiella (n=64)	Acinetobacter (n=50)	E.coli (n=32)	Pseudomonas (n=31)	Proteus (n=8)
Imipenem	18(28.13)	26(52)	7(21.88)	4(12.90)	0
Amikacin	39(60.94)	43(86)	18(56.25)	12(38.71)	1(12.5)
Piperacillin/ tazobactam	47(73.44)	39(78)	19(59.38)	9(29.03)	0
Gentamycin	55(85.94)	40(80)	18(56.25)	18(58.06)	0
Cefotaxime	37(57.81)	45(90)	24(75)	NT	0
Ceftazidime	38(59.38)	46(92)	24(75)	11(35.48)	0
Cefepime	37(57.81)	44(88)	23(71.88)	10(32.26)	0
Ciprofloxacin	58(90.63)	43(86)	23(71.88)	14(45.16)	0
Cefoperazone/Sulbactam	54(84.38)	26(52)	23(71.88)	13(41.94)	1(12.5)
Polymyxin B	2(3.13)	4(8.00)	0	0	NT

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Tigecycline	2(3.13)	6(12.00)	0	NT	NT
Colistin	NT	0	NT	0	NT
Levofloxacin	50(78.13)	35(70)	27(84.38)	7(22.58)	1(12.5)
Netilmicin	36(56.25)	44(88)	16(50)	11(35.48)	0
Amoxycillin /Clavulanate	47(73.44)	47(94)	24(75)	17(54.84)	0
Cotrimoxazole	51(79.69)	35(70)	24(75)	23(74.19)	0

***NT-Not Tested *Criteria as published by CLSI (6)**

DISCUSSION:

The dissemination of antimicrobial resistance among Gram negative organisms is of great concern as it increases the likely hood of prolonged hospital stay and cost of therapy. Thus the review of antibiotic resistance pattern and monitoring the use of antibiotics will help in better management of patients. The prevalence of antibiotic resistance varies in different study environment.

The predominant gram negative isolate in our study was Klebsiella spp, where as various studies by Zainab et al 2012 (7) and Morfin Otero et al 2012 (8) have reported E.coli as predominant organism. A similar study conducted by Ziab et al 2013 (9) reported Pseudomonas aeruginosa as predominant gram negative bacilli isolated from ICU.

The most frequent isolate from UTI was found to be E.coli. This is comparable to the studies conducted by Kritu panta et al 2013(10) and Sankarankutty J et al 2014(11).

Klebsiella spp was the most predominant organism isolated from respiratory specimen. A similar study conducted by patel et al 2012 (12) reported Klebsiella as most frequent isolate from respiratory tract infection followed by Pseudomonas spp and Acinetobacter .

This study shows a considerably higher prevalence of carbapenem resistance among Acinetobacter *spp* (52.00%) .A similar study conducted by mumtaz et al 2012 (13) have reported 79% resistance to Imipenem. These strains were also resistant to other antibiotics including Penicillins, quinolones, aminoglycosides and third generation cephalosporins. In our study 91 % of Acinetobacter were resistant to third generation cephalosporins,85 % isolates were resistant to aminoglycosides and 78 % were resistant to fluoroquinolones. The most effective drug against this multidrug resistant Acinetobacter *spp* was Colistin.

Carbapenem resistance in pseudomonas was found to be 12.90% in our study .In a study conducted by Rakhee et al2014 (14) 20.8% were resistant to Imepenem. This isolates also showed high degree of resistance to third generation cephalosporin and aminoglycoside. Resistance to Ceftazidime was found to be 35.48% in our study .Resistance to Amikacin and Gentamicin were found as 38.71% and 56.25%. Multidug resistant Pseudomonas have been reported by Pattanayak .C. et al 2013(15) and Ahmed hasanin et al (16).

Among Enterobacteriaceae, carbapenem resistance was found to be high in Klebsiella spp (28.13%), whereas 100% sensitivity to carbapenem was reported by Sheth et al 2012(17). A study reported by Aysen et al showed 12% Klebsiella isolates resistant to carbapenem .The percentage resistance of Klebsiella to third generation cephalosporins was found to be 59.00% in our study.A study reported by Maksum Radji et al 2011(18) have showed 70.00 % isolates resistant to third generation cephalosporins.Aysen et al 2006(19) have reported 13.1% E.coli isolates resistant to Imipenem and 84 % E coli and Klebsiella resistant to third generation cephalosporin. More than 90% E.coli and Klebsiella strains were found to be resistant to third generation cephalosporin by Parimal H patel et al(2013(20).

In the present study *E. coli* exhibited 56.25% resistance to Amikacin and Gentamicin. A similar study by Gunjal et al 2012 (21) have reported 28.10 % of E .coli isolates resistant to Amikacin and 48.20% resistance to Gentamicin, 60 .00% Klebsiella isolates were resistant to Amikacin and 80.00 % to Gentamicin .In the present study *Klebsiella e*xhibit 60.94% resistance to Amikacin and 85.94% to Gentamicin. Tigecycline and Polymyxin B were found to be most effective drugs against multidrug resistant Enterobacteriaceae except Proteus.

CONCLUSION:

Infection due to MDR microorganisms is a rising problem, especially in the ICU where even sensitive pathogens cause prolonged hospital stays, higher health care cost, and increased mortality. Therefore, constant evaluation of current practice on basis of trends in multidrug resistance and antibiotic consumption patterns are essential.

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