A CROSS-SECTIONAL STUDY TO DETERMINE THE PROFILE AND ANTIBIOTIC RESISTANCE PATTERN OF GRAM NEGATIVE BACILLI ISOLATED FROM INTENSIVE CARE UNIT PATIENTS IN A TERTIARY CARE HOSPITAL IN AHMEDNAGAR, MAHARASHTRA

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Abstract

The present study was conducted to track down commonest gram negative bacilli along with their pattern of resistance to antibiotics from various clinical specimens, from patients admitted to intensive care unit. A total of 182 clinical specimens were analyzed using standard techniques. Out of 182 specimens 105 were culture positive, whereas the remaining 77 were culture negative. From 105 culture positive specimens 65 (61.90%) showed growth of gram negative bacilli, remaining 40 (38.09%) with growth of gram positive organisms. In this study *E .coli* was found to be the predominant isolate with 44.61% rate of isolation, followed by *Klebsiella pneumoniae* 15.38%, rate of isolation of *Proteus mirabilis, Citrobacter fruendii* each accounting for 9.23%. *Pseudomonas aeruginosa* 7.69%, *Proteus vulgaris* 3.07%, *Acinetobacter species* and *Salmonella typhi* accounted for 1.53 % each. To conclude, aerobic gram negative bacilli were predominant clinical isolates from patients admitted to intensive care unit. Amikacin and gentamicin were found to be effective antibiotics against gram negative bacilli in this particular setting.

Keywords: *E. coli, Klebsiella, Pseudomonas, Proteus, Acinetobacter,* Drug resistance, antimicrobial sensitivity test

1. Introduction:

Infection and antibiotic resistance are important public health issues. One of the major problems worldwide is the increase in antibiotic-resistant strains of microorganisms, mainly bacteria in hospital environment. The "Nosocomial Pathogens" and the drug resistant strains in the community are difficult to control without considerable resources and expenditure.[1] Multidrug resistant strains of gram negative bacilli continue to spread in hospitals causing therapeutic problems in most parts of the world, particularly in developing countries where isolation facilities for patients with resistant organisms are often inadequate.[2] Moreover, during the last few years, the increase in antibiotic resistance has compromised the selection of empirical treatment.[3] The knowledge of likely prevalent strains along with their antimicrobial resistance pattern will help in better management of patients.[4]

The present study was therefore conducted with an aim of tracking the commonest gram negative bacilli along with their resistance rate, from various clinical specimens from patients admitted to the intensive care unit of a tertiary care hospital at Ahmednagar, Maharashtra, India.

2. Materials and Methods:

The present study was conducted at the Microbiology Department of Padmashree Dr. Vitthalrao Vikhe Patil Foundation's Dr. Vikhe Patil Memorial Hospital and Medical College, Ahmednagar, Maharashtra, India, which is a tertiary care hospital.

Clinical specimens were collected from patients of all age groups and genders admitted to the intensive care unit.

Pure or mixed growth from one subject and consecutive samples from new subjects were included in the study.

Various clinical specimens like blood, urine, pus, sputum, stool, CSF, and other body fluids were collected aseptically according to standard techniques.[5] All the specimens were subjected to direct microbial observation using Gram's staining method. Specimens were immediately cultured under aseptic condition

on sterile blood agar and Mac Conkey's agar and incubated aerobically at 37 C for about 18-24 hrs. period. Blood culture was followed according to standard methods.[6] Isolates were identified by morphology, cultural characteristics and biochemical identification tests using standard methods.[7, 8] Antibiotic susceptibility testing was done by disc diffusion method.[9] The following antibiotics were tested.

Amikacin (30µg), gentamicin (10µg), ampicillin (30µg), cefotaxime (30µg), cefuroxime (30µg), and ciprofloxacin (05µg). Antibiotic discs were procured from Hi-media. For quality control of disc diffusion method, ATCC control strains of *E. coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 were used.

3. Results:

A total of 182 specimens were analyzed. Out of 182 specimens 105 were culture positive, whereas the remaining 77 specimens showed no microbial growth after incubation period.

From 105 culture positive specimens 65 (61.90%) were isolates of gram negative bacilli while remaining 40 (38.09%) were gram positive organisms.

Aerobic gram negative bacilli were found to be the predominant isolates from various clinical specimens in patients from the intensive care unit. (Shown in Table 1,2 3 & 4)

4. Discussion:

Aerobic gram negative bacilli remained the predominant (61.90%) isolates from various clinical specimens obtained from patients admitted to the intensive care unit. This was in consistence with other workers like Gagneja *et al.*[4] and Kumari *et al.*[10] Gagneja isolated 91.07% gram negative bacilli and 8.92% gram positive organisms and Kumari *et al.* reported 85.9% gram negative bacilli as compared to 6.2% *Staphylococcus aureus.* Both the workers reported gram negative bacilli more predominantly as compared to the gram positive organisms.

Escherichia coli was isolated from 44.69% of the cases, which is in contrast with other workers like Gagneja *et al.*[4] who reports 9.25% in year 2008–2009 and Kumhar *et al.*[11] reported 4.6% *Escherichia coli* among other gram negative bacilli. Differences could be there between the present study and Gagneja *et al.*[4] and Kumhar *et al.*[11] because of natures of specimens. Gagneja *et al.*[4] reported gram negative bacilli only from the specimens obtained from lower respiratory tract infections and Kumhar *et al.*[11] reported gram negative bacilli only from blood stream infections from the NICU patients.

Isolation of *Klebsiella pneumoniae* was 15.38%, which is well in accordance with Gagneja *et al.*[4] who reports 16.7%.

As far as age is concerned, maximum isolates were obtained from the age group of 31-40 yrs. followed by the age group of > 50 yrs. and minimum from age group of 11-20 yrs. The high

rate of isolation of gram negative bacilli from the age group to 31-40 yrs. is in accordance with Gagneja *et al.* who reported 42.15 % gram negative bacilli from the age group of 18-64 yrs. In our study, a higher rate of isolation (40.42 %) from age group of >50 yrs., as compared to the other age groups may be due to the risk of infection with increasing age.

The present study reports total resistance to ampicillin (100 %) which is well in range with the study of Gagneja *et al.*[4], who reported 97.56% resistance to ampicillin by the gram negative bacilli in 2004-2005 and 97.19 % resistance in 2005-2006.

This is also in well accordance with Kumari *et al.*[10], who reported 98.7% resistance to ampicillin in *Klebsiella species* and 100% resistance in *E. coli* isolates from tracheal specimens and 100 % resistance in both *E. coli* and *Klebsiella species* isolated from bronchial specimens.

Total (100%) resistance was also exhibited against cefotaxime which is again close to the finding of Kumari *et al.*[10], who reported 90.5% resistance in *Klebsiella species* and 97.7% resistance in *E. coli* isolates obtained from the tracheal aspirates and 92.4 % resistance to both

E.coli and *Klebsiella species* isolated from bronchial specimens. Resistance to cefotaxime (100%) is in contrast to Gagneja *et al.*[4], who reported 71.95% resistance in 2004-2005 and 70.09% resistance in 2005-2006. High resistance to ampicillin and cefotaxime must be probably because of prolonged over exposure of bacteria to these antibiotics.

In the present study *E. coli* exhibited 24.10% resistance to amikacin and 48.20% resistance to gentamicin. Kumari *et al.*[10] reported higher resistance of 38.5% to amikacin and 92.4% to gentamicin in *E. coli*.

In the present study *Klebsiella pneumoniae* exhibit 60% resistance to amikacin and 80% to gentamicin, whereas Kumari *et al*.[10], reported lower resistance of 23.1% to amikacin and higher resistance of 100 % to gentamicin.

Overall, amikacin and gentamicin showed better activity against both, *E. coli* and *Klebsiella pneumoniae* isolates.

Differences reported in the present study and studies of other workers mentioned could be probably because of variations in the settings and use of different antibiotic policies in the respective hospitals.

To conclude, gram negative bacilli were the predominant isolates from various clinical specimens collected from the intensive care unit patients, *E. coli* and *K. pneumoniae*, being the most common isolates. Amikacin and gentamicin were found to be effective antibiotics against these isolates.

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Table No. 01 – Profile of Gram negative bacilli from clinical specimens.

Sr. No.	Gram negative bacilli isolates	Total number of isolates	Percentages (%)
1	*Escherichia coli*	29	**44.61**
2	*Klebsiella pneumoniae*	10	**15.38**
3	*Citrobacter fruendii*	06	**9.23**
4	*Proteus mirabilis*	06	**9.23**
5	*NFGNB*	05	**7.69**
6	*Pseudomonas aeruginosa*	05	**7.69**
7	*Proteus vulgaris*	02	**3.07**
8	*Acinetobacter species*	01	**1.53**
9	*Salmoneall typhi*	01	**1.53**
	Total	**65**	**100**

NFGNB: Non - fermenter gram negative bacilli other than Pseudomonas species.

E. coli (44.61%) was found to be the predominant isolate, followed by *Klebsiella pneumoniae* (15.38%). The other agents were less common.

Table No. 02 - Gender wise distribution of isolates.

Sr. No.	Particulars	No. of subjects	No. of isolates from subjects.
1	No. of females with single isolate each	15	15
2	No. females with 2 isolates each	03	06
3	No. of females with 3 isolates each	00	00
4	No. of male with single isolate each	31	31
5	No. of male with 2 isolates each	05	10
6	No. of males with 3 isolates each	01	03
	Total	**55**	**65**

Out of the 65 gram negative bacilli, 21 isolates were recovered from 18 females and 44 isolates were recovered from 37 males, 2 isolates each were obtained from 3 females and 5 males and 3 isolates were obtained from a single male subject.

Table No. 03 - Age wise distribution of the gram negative bacilli (GNB) isolates.

Sr. No.	Age groups (in years)	Total no. of patients in the respective age group	No. of isolates obtained	Percentages (%)
1	>10	8	3	37.50
2	11-20	14	1	7.14
3	21-30	20	7	35.00
4	31-40	25	11	44.00
5	41-50	21	5	23.80
6	>50	94	38	40.42
	Total	**182**	**65**	

Maximum rate of isolates are obtained from the age group of 31-40 yrs (44.00%), followed by the age group of > 50 yrs (40.42 %) and least from the age group of 11-20 yrs. (7.14%).

Table No. 04 Resistance rate (%) to tested antimicrobials for the most common Gram negative bacilli isolates from the specimens.

Sr. No.	GNB	No. of isolates (n)	Antibiotic tested (disc of antibiotic)					
			AK (30µg)	GM (10µg)	AM (30µg)	CU (30µg)	CI (30µg)	CF (05µg)
1	***E. coli***	n = 29	24. 10	48.20	100	89.60	100	93.10
2	***K. pneumoniae***	n = 10	60.00	80.00	100	70.00	100	80.00

GNB - Gram Negative Bacilli, *E. coli* - *Escherichia coli*, *K. pneumoniae* - *Klebsiella pneumoniae.*

AK - Amikacin, GM - Gentamicin, AM - Ampicillin, CU - Cefuroxime, CI - Cefotaxime, CF - Ciprofloxacin.