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A PROSPECTIVE OBSERVATIONAL ANALYSIS OF ANTIMICROBIAL DRUG RESISTANCE IN GRAM NEGATIVE BACTERIA ISOLATED FROM INTENSIVE CARE UNITS OF A TERTIARY CARE HOSPITAL

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ABSTRACT Intensive care units are becoming the focal point of hospital acquired infection and antimicrobial resistance development. Gram negative bacilli contribute significantly to above problems. This study aimed to detect the antibiotic resistance pattern of gram negative bacilli in patients admitted to various ICUs of a tertiary care hospital. This is a prospective observational study conducted in the Department of Microbiology, Subharti Medical College, Meerut from April 2018 to December 2018. The study included 320 gram negative bacilli isolated from samples obtained from patients admitted in ICUs. Antibiotics sensitivity testing was done by standard protocol. Out of 320 gram negative bacilli isolated, predominant was Klebsiella spp. followed by E.coli and Pseudomonas. Percentage of ESBL and carbapenemase producers was 48 and 36, respectively. Maximum resistance was shown for pipperacillin, ampicillin, amoxicillin- clavulanic acid, ampicillin - sulbactum, ceftriaxone, aztreonam and ciprofloxacin. The current study showed that the rate of multi dug resistance in gram negative bacilli is quite high. To monitor this issue an effective antibiotic stewardship program should be formulated.

KEYWORDS : Intensive care units, antimicrobial resistance, Gram negative bacilli

INTRODUCTION

Currently the world is facing a devastating problem i.e. rampant spread of multidrug resistant superbugs. Their main epicentre is intensive care units, as the patient admitted there are in critical condition and most of them have associated co-morbidities with declining immunity. [1]

These multidrug resistant bacteria are becoming the main reason for increased morbidity and mortality among the vulnerable patients admitted in ICUs, imposing serious challenges to health care workers. It has been said that mortality rate is twice among the infected ICU patients compare to non infected patients.[2]

Numerous worldwide surveillance studies have detected various bacterial pathogens and their resistance pattern in ICUs.[3, 4] Production of extended spectrum beta lactamases (ESBL), AmpC beta - lactamase or carbapenemase lead to development of multiple drug resistance among microorganisms.[5]

Administration of unjustifiable antimicrobials imposes selective pressure on bacteria resulting in the growth of previously susceptible strains which has now acquired resistance or there can be over growth of organisms which are intrinsically resistant. Plasmids facilitate transfer of resistance among the bacteria of same genera as well as between organisms deferent genera.[6]

The availability of regional antimicrobial data is of prime importance to implement a productive and practical treatment approach against infectious agents. This might also help in restricting the MDR organisms at the regional level.[3, 7] Also the speed at which antimicrobial resistance is spreading; it exceeds the pace of development of new antibiotics to tackle these MDR organisms.[8]

Such local surveillance should be done at regular interval for several reasons like, change in the prevalence pattern of MDR pathogens, patient populations catered by the hospital, type of patient admitted in ICUs, antimicrobials administered etc.[5]

This study aims to highlight the emergence and spread of multi drug resistant bacteria in the ICUs of a tertiary care hospital and their antimicrobial resistance pattern.

MATERIAL AND METHODS:

Study design -

This was a prospective observational study conducted in the department of Microbiology in a tertiary Care hospital in Meerut, Uttar Pradesh. The study was conducted from April 2018 to December 2018. Patients admitted to various Intensive Care Units (ICUs) of Chhatrapati Shivaji Subharti Hospital were included in the study.

Inclusion criteria -

All samples received in the Clinical Microbiology laboratory during the study period from patients admitted in various Intensive Care Units (ICUs) of Chhatrapati Shivaji Subharti Hospital.

Exclusion criteria-

Patients who have received antimicrobial therapy prior to culture were excluded from the studies. Blood samples were not included in the study.

Ethics approval-

The study has been approved by institutional ethical and Research committee of Subharti Medical College.

Methodology -

A total of 1800 samples including sputum, pleural fluid, pus, urine, tracheal aspirate, spinal fluid and other body fluids were included in the study. The samples were carefully and aseptically transferred to the laboratory at the earliest for further examination. Samples were processed as per the standard protocol.

Samples were subjected to aerobic bacterial culture on blood Agar, chocolate agar and MacConkey agar plate. Urine samples were cultured on cystine-lactose-electrolyte-deficient-agar medium. Pus and fluid samples were also inoculated in brain heart infusion broth for enrichment. All culture plates and brain heart infusion broth were incubated at 37º C for 48 hours. Colonies observe next day on the culture media were identified using biochemical test like catalase, oxidase, Indole, urease, citrate, triple sugar iron agar test. Antibiotic susceptibility test of the isolate was done on mueller hinton agar medium by disc diffusion technique according to clinical laboratory standard Institute (CLSI) guidelines.

Antibiotics used were Ampicillin(10µg), Piperacillin(100µg), Pipercillin/Tazobactam(100/10µg), Ampicillin/ Sulbactum(10/10µg),Cotrimoxazole(1.25/232.75µg), Tetracycline(30µg), Amoxicillin-Clavulanic acid (20/10µg), Chloramphinicol (30µg), Gentamicin (10 µg), Ciprofloxacin (5µg), Cefixime(5µg), Ceftazidime(30µg), Ceftriaxone(30µg), Aztreonam(30µg), Cefepime(30µg), Amikacin(30µg), Tobramycin(10µg),

Ertapenem(10µg), Meropenem(10sµg), Imipenem(30µg),Cefotaxime/Clavulanic acid(30/10µg), Cefotaxime(30µg), Ceftazidime/Clavulanic acid(30/10µg).

Minimum inhibitory concentration was detected for Colistin (10 µg) using broth dilution test. Detection of extended spectrum beta-lactamase production was done by double disk diffusion method. Isolates showing resistance to ceftazidime (30 µg), aztreonam (30 µg), ceftriaxone (30 µg) were put to double disk synergy test using ceftazidime and ceftazidime-clavulanic acid (30/10 µg). A difference of more 5mm in the diameter of inhibitory zones of both disks was considered as positive for ESBL production. Detection of carbapenemases was done using Imipenem (30 µg) as per clinical laboratory standard Institute recommendations.

RESULT

A total of 1800 samples were processed during the study period. Out of which 320 (17.78%) samples showed gram negative bacilli growth. Samples from 196 male and 124 female patients showed gram negative bacteria growth. Gram negative bacteria were isolated thorough out the study and maximum numbers isolated were seen during the month of August and July.

Table 1: Age wise distribution of patients with Gram Negative Bacteria growth (n= 320)

S. no.	Age(in years)	No. of Isolates	Percentage
1.	<10	47	14.7
2.	11-20	15	4.8
3.	21-30	20	6.2
4.	31-40	27	8.4
5.	41-50	43	13.4
6.	51-60	64	20
7.	> 61	104	32.5
Total		320	100

Table 2: ICUs wise distribution of Gram negative bacteria (n=320)

ICUs	Isolates	Percentage
SICU	139	43.3
MICU	78	24.37
RICU	45	14.2
SP.ICU	33	10.3
PICU	13	4.07
NICU	6	1.87
BICU	5	1.58
CCU	1	0.31
p value=<0.0007(S)		

SICU- surgical ICU, MICU- medical ICU, RICU- respiratory ICU, SP. ICU- special ICU, PICU- pediatric ICU, NICU- neonatal ICU, BICU- burn ICU, CCU- cardiac care unit

Table 3 : Bacteriological Profile of Gram negative isolates

Bacteria Isolated	Number of Isolate	Percentage (%)
Klebsiella spp.	138	43.2
E.coli	103	32.1
Pseudomonas spp.	52	16.3
Citrobacter spp.	15	4.6
Acinetobacter spp.	12	3.7
Total	320	100

Table 4: Percentage resistant of Gram negative bacteria isolated from ICUs samples to various antibiotics (%)

Gram Negative Bacteria	Klebsiella Spp.	E.coli Spp.	Pseudomonas Spp.	Citrobacter Spp.	Acinetobacter Spp.
AMP	99	97	Nt	Nt	Nt
PI	99	99	74	74	85
AMC	98	99	Nt	Nt	Nt
A/S	90	95	Nt	Nt	Nt
PIT	75	90	45	48	74
TE	90	77	Nt	Nt	Nt
COT	90	90	Nt	Nt	70
CIP	95	95	82	82	70
CFM	97	96	Nt	Nt	Nt
CAZ	95	95	64	68	77
CTR	98	98	Nt	Nt	75
AT	95	95	70	70	Nt
CPM	90	90	50	50	Nt
GEN	70	35	72	72	75
AK	70	35	68	68	75
TOB	73	40	65	65	70
C	70	25	Nt	Nt	Nt
ETP	50	40	Nt	Nt	Nt
MRP	51	40	45	45	78
IMP	50	40	37	37	75
CL	0	0	0	0	0

Nt = Not tested,
AMP- Ampicillin, PI- PiperacillinAMC- amoxicillin/clavulanic acid, A/S- ampicillin-sulbactum, PIT- itperacillin/tazobactam, TE-Tetracycline, COT-Cotrimoxazole,CIP-Ciprofloxacin, CFM-Cefixime, CAZ-Ceftazime, CTR- Ceftriaxone, AT- Aztreonam , CPM-Cefepime, GEN-Gentamicin, AK-Amikacin,TOB-Tobramycin, C-Chloramphenicol, ETP- Ertapenem, MRP-Meropenem, IPM- Imipenem, CL- Colistin.

DISCUSSION

Patients admitted in ICU are in critical condition leading to immediate administration of antibiotics without waiting for culture and sensitivity report. That is why antimicrobial resistance is a critical issue in ICU setting. It is of prime importance to reduce antimicrobial resistance in ICUs. Many studies support that the increased usage of antimicrobials in ICUs for prophylactic and therapeutic purposes has led to a constant growth of antimicrobial resistance among bacteria. This has indirectly resulted in enhanced heath care cost and increased mortality. The incidence rate of antibiotic resistance pattern in ICU is 23.7 infections per thousand patient days.[9]

Patient from all age group were included in this study. Maximum numbers of samples were from patient above 60 years. This could be due to multiple factors including declining immunity in old age and associated co-morbidities.

It was observed that out of 320 isolates obtained from the ICUs there was a male predominance (61%) compared to female patients (39%). This data is almost similar to a study carried out by Bhatia A et al., where they detected 55 % male patients and 45 % female.[10] The relative higher cases in males may be due to their greater participation in outdoor activities and hence higher chances of acquiring infections.

The most common infecting organism isolated in our study was *Klebsiella spp*, next were *E. coli, Pseudomonas, Citrobacter* species *and Acinetobacter* species. This is in accordance with the study conducted by Bhatia A et al. They isolated *Klebsiella pneumoniae* in their study done in Dehradun (Uttarakhand), as the most common gram negative bacilli followed by *E. coli* and *Acinetobacter*.[10]

Similarly Negm EM et al. and W. Mulu et al., identified *Klebsiella pneumoniae* as predominant isolate in their study conducted in Egypt and Ethopia, respectively.[11,12]

In another study carried out in Haldia (West Bangal), A. Anand found out that predominant gram negative bacilli isolated from respiratory ICU was *Pseudomonas aeruginosa* followed by *Klebsiella pneumoniae*.[9] Whereas Savanur S et al., detected *E. coli* as a predominant isolate among gram negative bacilli followed by *Acinetobacter* species, *Klebsiella* species., *Pseudomonas* secies and *Proteus* species in Dharwad, Karnataka[13]

In our study we found out high antimicrobial resistant rate in most of the gram negative bacilli. Maximum resistance was for ampicillin (97 – 99%), followed by amoxicillin/clavulanic acid (96 - 99 %), Piperacillin (74-99 %), ampicillin-sulbactum (75 to 95%), Ceftriaxone (98%), Ceftazidime(64 – 95%), Ciprofloxacin (70 to 95%), Cotrimoxazole (70 –90%), Tetracycline (77 to 90 %), piperacillin/tazobactam (45 - 90%), Cefixime (95 - 97%), Aztreonam (70–95%), Cefepime (50–90%), Tobramycin (40 to 73%), Amikacin (35–70 %),Gentamicin(35-70%), Chloramphenicol (25- 70%), Meropenem (40 – 78%), Ertapenem (40 -50%), Imipenem (37 - 75%).

All our isolates were sensitive to colistin. Various national and international studies have shown quite high degree of antimicrobial resistance among gram negative bacilli isolated from ICU patient. In one of the study conducted by Bhatia A et. al., immense resistance among gram negative bacilli was detected. They detected up to 100 % resistance to various antimicrobials like piperacillin-sulbactum, aztreonam, ceftriaxone, cefepime ciprofloxacin, piperacillin/tazobactam, imipenem, meropenem, and ertapenem. In their study they found 22% resistance to colistin.[10]

Savanur SS et al., detected comparatively less resistance in ICU setting. They found out sensitivity for amikacin 18 – 70%, gentamicin 21 - 77%, ampicillin 0– 37 %, cefepime 60 - 76%, ceftriaxone 62 – 96 %, ceftazidime 60–96 %, ciprofloxacin 21 – 91 %, cotrimoxazole 40 – 92%, imipenem 12-47%, piperacillin/tazobactam 40 – 84 %, aztreonam 0 – 12%.

Faheem N, detected a very high over all resistance to the commonly used antibiotics. The least frequency of resistance was recorded against amikacin, imipenem, meropenem and there was a 100 % susceptibility to colistin in concordance with our study.[14]

In the current study, resistant to beta lactam and beta lactam – beta lactamase inhibitor combination was frequently found among the gram negative bacilli. The ESBL and Carbapenemase producers were more among *Klebsiella* species as compared to *E.coli, Pseudomonas*, *Citrobacter* and *Acinetobacter*. P value obtained for ESBL was p=0.0007 and carbapenemase p = 0.0003, it showed that the results obtained were significant.

Mulu W et al., found out *Pseudomonas aeruginosa* as the most frequent ESBL producer followed by *E. cloacae, K pneumoniae and E. coli* where as among carbapenemase producers, klebsiella pneumoniae and E. cloacae were the predominant organism.[12]

A high level of resistance seen in the gram negative bacilli in the current study is in accordance with the various results published by other researchers. Presence of Beta lactamases can contribute to such a high resistance among gram negative bacilli. In India availability of antibiotics over the counter leading to self medication, improper dosage and for a shorter duration contributes extensively to antimicrobial resistant.

Multidrug drug resistant gram negative bacilli load is much more in intensive care unit compared to rest of the hospital department because of unconstrained usage of antibiotics.[15]

The high resistant rate in gram negative bacteria in our study provides a general over view of the present antimicrobial resistance scenario in the hospital under study. Immediate actions have to be taken to prevent its augmentation.

Advance knowledge of antibiotic susceptibility pattern can help clinicians to improve the prescribing patterns and also in taking decisions to restrict and reduce usage of certain antibiotics. Broad spectrum antimicrobials should be shifted to targeted antimicrobial on immediately receiving the culture sensitivity report.

Limitation:

Gram positive bacteria, anaerobic bacteria and fungal pathogens were not including in this study even though they are commonly isolated from ICU setting. Their inclusion in the study would have given a brooder and comprehensive information about antimicrobial resistance in the ICU.

CONCLUSION:

To avert the emergence of drug resistance among bacteria, constitution of a workable antibiotics policy is the pressing priority. This is required at hospital level, state level as well as at national level. This will be useful in controlling tendency of over prescribing antimicrobials among clinicians. Stern measures should be taken by the Government to curtail over the counter sail of antimicrobials. Regular training of health care workers on the above mention issues is incomparable.

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Ethical approval : The study was approved by the institutional ethical committee. (SMC/IEC/2018/204/2615)

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