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Original Research Article

Antibiotic susceptibility pattern of gram-negative bacterial isolates with special mention on colistin resistance from Intensive Care Unit of a tertiary care hospital: a prospective study assessing the impact of microbial resistance on clinical outcomes

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ABSTRACT

Background: The frequent use of broad-spectrum antibiotics in ICU leads to increased rates of antimicrobial resistance and occurrence of multidrug-resistant (MDR) micro-organisms. The aim of this study was to evaluate the antimicrobial resistance pattern and colistin susceptibility among bacterial isolates from ICU patients.
Method: It is a prospective study with 70 nonrepetitive isolates from ICU samples. The clinical data was obtained from the department records. The gram-negative bacterial isolates were identified by conventional biochemical tests. The antimicrobial susceptibility was determined by Kirby-Bauer disc diffusion method. ESBL producers were detected by double disc diffusion test using ceftazidime, cefotaxime alone and in combination with clavulanic acid. MBL detection was done by imipenem+ EDTA combined disc diffusion test. Colistin sensitivity was determined by broth microdilution according to CLSI guidelines.
Results: Out of 70 culture positive specimens. The most common gram-negative bacteria isolated from the samples was *Acinetobacter* spp. (41%), followed by *Klebsiella* spp. (20%). Among these 45% were MBL producers, 38.5% were ESBL producers and 14% were both ESBL and MBL producers. Colistin resistance was present among 5.7% isolates in ICU.
Conclusions: Non-fermenters were the most common agent causing ICU infections. An alarmingly high rate of resistance to antibiotics especially to colistin in ICU-acquired infections, necessitates new therapeutic strategies to prevent the emergence and control of antimicrobial resistance.

Keywords: Antimicrobial resistance, ICU, Colistin resistance

INTRODUCTION

The Intensive Care Unit (ICU) often is called the epicenter of infection. It caters to an extremely vulnerable population which is at an increased risk of becoming infected due to usage of multiple invasive interventions, factors causing loss of anatomical barriers and drugs which interfere with the normal microbiota and immunity.[1]

Antimicrobial resistance (AMR) has emerged as one of the most important determinants of outcome for patients in the ICU.[1] AMR is most commonly due to the administration of inadvertent antimicrobial treatment. This leads to prolonged hospitalizations, treatment failures and increase in financial burden for the patients.[3] There is an immediate need for implementation of infection control and public health interventions specially to curb AMR

Bacteria have developed multiple resistance mechanisms. Several gram-negative bacteria are major examples of MDR organisms where pan-resistance is now being found due to the presence of integron gene cassettes with multiple resistance genes that encode one or more β-lactamases, aminoglycoside modifying enzymes, fluoroquinolone resistance determinants, tetracycline resistance, and resistance to disinfectants.[8]

Carbapenems were once effective and reliable antimicrobials for the treatment of extended spectrum beta lactamase (***ESBL***) producing oganisms. Currently, serious concerns due to the global spread of carbapenem-resistant bacteria are raised and there are only very few compounds available as a treatment choice for MDR pathogens. All these have made Colistin the last treatment option for infections by carbapenemase producing bacteria.

Colistin is a polymyxin group of antibiotics which acts by interacting with lipopolysaccharides on the outer membrane of gram-negative bacteria and causes injury to membrane leading to bacterial death. Colistin resistance results from two mechanisms: Chromosomal defects or plasmid resistance. Chromosomal mutations occur in the PmrA/ PmrB and PhoP/ PhoQ encoding genes leading either to lipid A molecule modifications or even loss. These mutations are related to colistin usage.[5] Though, colistin resistance is present without prior exposure to colistin, due to the presence of plasmid mediated mcr-1 gene encoding phosphoethanolamine transferase enzyme leading to transfer of phosphoethanolamine to lipid A; confers colistin resistance.[5]

The high prevalence of resistance to the empirical antibiotic regimens in ICU's highlight the need for modifying the empirical treatment regimens considering the most effective antibiotics for gram negative bacteria according to the local antibiotic policy.

Objective

This study aims at establishing the common pathogens isolated from ICU infections in the hospital and to enumerate the presence of various resistance patterns among these isolates. A special emphasis on the presence of colistin resistance among these isolates from ICU acquired infections was established.

METHODS

This is a cross sectional prospective study consisting of 70 consecutive, nonrepetitive clinical isolates from blood, urine, sputum, endotracheal tube (ET) aspirate, catheters, and wound swabs, broncho-alveolar lavage (BAL) fluid and pus from patients admitted to all ICU of K. R. hospital, Mysore collected over a period of 6 months from November 2021 to January 2022. All the isolates were identified on the basis of gram staining, colony morphology and standard biochemical tests.[8]

Antibiotic susceptibility testing[2,25]

Mueller-Hinton agar medium was inoculated with a peptone suspension of isolated gram-negative bacteria equivalent to McFarland 0.5 turbidity standards. Antibiotic discs were applied on the surface of agar. After 16-18 hours of aerobic incubation at 37°C, the antimicrobial susceptibility of gram-negative bacteria was determined by the Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standard Institute (CLSI) guidelines 2021. The isolated aerobic gram-negative bacteria were categorized to be resistant, intermediately susceptible, depending on the size of inhibition zone.

Detection of ESBL production[2,25]

This was performed by phenotypic method of confirmatory test as per the recommendations of CLSI 2021. The ceftazidime and cefotaxime (CA/CE) (30 µg) discs alone and in combination with clavulanic acid (CAC/CEC) were used. The discs were placed at a distance of 20mm to each other on the Muller Hinton agar medium inoculated with a peptone suspension of isolated gram-negative bacteria taken from a 24-hour growth from blood agar plate adjusted to an equivalent of the McFarland 0.5 turbidity standards. The difference in inhibition zones displayed around the (CA/CE) as well as the (CAC/CEC) disks were compared after the 16- 18 hrs of incubation at the 37°C. The difference of ≥5 mm between the inhibition zone diameter of the (CAC/CEC) disk and that of (CA/CE) only disk was considered to be a positive for the presence of the ESBL production.

Detection of metallo-beta-lactamase (MBL) production [2,25]

This was performed by phenotypic confirmatory test as per the recommendations of CLSI 2021. Two imipenem (I) disks were placed on the surface of the agar at a distance of 20 mm to each other on the Muller Hinton agar medium inoculated with a peptone suspension of isolated gram-negative bacteria equivalent to McFarland 0.5 turbidity standards taken from a 24-hour growth from blood agar, 5 µL of 750 μg/mL EDTA solution is then added to one of the imipenem discs. The inhibition zones displayed around the I and the I+EDTA disks were compared after 16 to 18 hrs of aerobic incubation at 37°C. The difference of ≥7 mm between the inhibition zone diameter of the I+EDTA disk and that of imipenem (I) only disk was considered to be a positive for the presence of MBLs.

Detection of colistin resistance[26]

Minimum Inhibitory Concentration (MIC) of colistin was detected broth-microdilution method according to CLSI guidelines as follows-The primary drug stock solution of colistin was prepared by weighing 10 mg of colistin sulfate powder with a potency of 765 µg/mg. To achieve

a concentration of 1000 µg/ml (1 mg/ml), 7.65 ml of the autoclaved distilled water was added, following the potency calculations (7650 µg /7.65 ml=1 mg/ml or 1000 µg/ml).

From the primary stock solution, various working stock solutions of colistin were prepared. Starting with a concentration of 64 µg/ml, 64 µl from the primary stock solution was added to 936 µl of autoclaved cation-adjusted Muller Hinton Broth (CA-MHB) medium in microcentrifuge tubes.

For the preparation of dilutions of colistin, 500 µl of the 64 µg/ml working stock solution was added to 500 µl of MHB medium in microcentrifuge tubes. Subsequent twofold serial dilutions were made in 9 microcentrifuge tubes, resulting in drug concentrations of 32 µg/ml, 16 µg/ml, 8 µg/ml, 4 µg/ml, and down to 0.125 µg/ml.

To set up the 96 well round-bottom microtiter plate, the following steps were performed: In each well of columns 1 to 10, 50 µl of CA-MHB broth was added. In column 11, 75 µl was added, and in column 12, 100 µl of CA-MHB broth was added, serving as the growth control and media control, respectively.

Starting from column 1, 25 µl of the corresponding colistin dilution (ranging from 64 µg/ml to 0.125 µg/ml) was added to each well. Column 11 contained only media and bacterial inoculum, while column 12 contained only 100 µl of media. The total volume in each well were 100 µl.

To prepare the inoculum, a standardized suspension with a turbidity equivalent to 0.5 McFarland was prepared using the direct colony suspension method, resulting in a concentration of approximately $1.5x10^8$ CFU/ml. This suspension was further diluted 1:75 by adding of autoclaved CA-MHB medium. From this diluted suspension, 25 µl was added to each well in columns 1 to 11, which already contained 75 µl (50 µl MHB + 25 µl antibiotic), resulting in a bacterial concentration of approximately 5×10^4 CFU/well.

The microtiter plates were then incubated at 35±2°C for 16 to 20 hours in an ambient air incubator within 15 minutes of adding the inoculum. To prevent drying, the microdilution tray was sealed with a tight-fitting plastic cover before incubation. Quality control was performed using Escherichia coli ATCC25922 and *Pseudomonas aeruginosa* ATCC27853 strains.

Interpretation of results

MIC of colistin is taken as the lowest concentration of colistin that completely inhibits visible growth of the organism in the microdilution wells as detected by the unaided eye and interpretation values were given according to CLSI guidelines 2021.

RESULTS

In the 70 ICU isolates 50 (71.4%) were male patients and 20 (28.5%) were female patients. Maximum number of patients belonged to age group of 40-50 years (57%) (Figure 2).

Among 70 ICU isolates 46 (65%) were blood samples, 13 (19%) were pus samples, 7 (10%) sputum samples and 4 (6%) urine samples. Majority of the samples were from medical ICU (RICU and ICU) (44.2%), pediatric ICU (35.7%), followed by surgical related ICU's (20%).

The variety of gram negative organisms isolated from the ICU samples have been depicted in the (Figure 1).

The most prevalent organisms among neonatal ICU and pediatric ICU was *Acinetobacter* spp (46%) and (91%) respectively. In medical ICU *Klebsiella* spp (66%) was most prevalent.The Surgical ICU also had 38% predominance of *Klebsiella* spp. In Respiratory ICU's non-fermenters (*Pseudomonas* spp and *Acinetobacter* spp.) were more prevalent (36%).

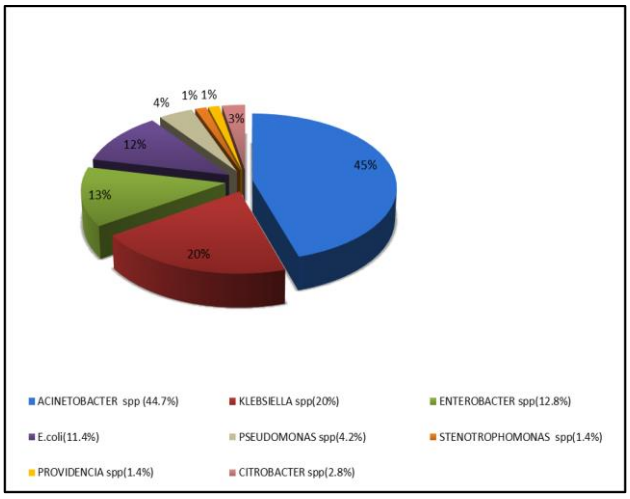

Figure 1: Distribution of gram-negative pathogens in the ICU.

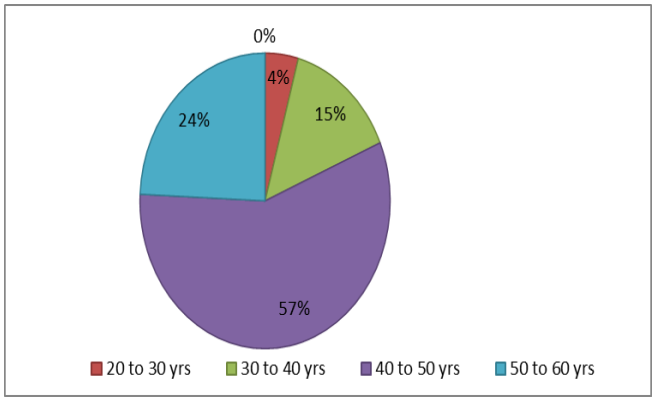

Figure 2: Age distribution of patients admitted to ICU.

Susceptibility pattern among isolates

In the total isolates 34/70(48%) isolates belonged to the family *Enterobacterales* and 36/70 (51%) belonged to the nonfermenting group of gram negative bacilli.

All the *Enterobacterales* (n=34) showed complete resistance to ampicillin, *E. coli* (n=7) showed highest resistance to cefotaxime, ciprofloxacin followed by cotrimoxazole and gentamicin but was sensitive to imipenem and piperacillin and Tazobactam. The *Enterobacter* spp. (n=9) showed highest resistance to cefotaxime, gentamicin followed by piperacillin tazobactam and amikacin but was sensitive to imipenem, cotrimaxazole and fluoroquinolones. The *Klebsiella* spp. (n=15) showed resistance to ciprofloxacin, piperacillin/ tazobactam, cefotaxime, cotrimaoxazole, gentamicin followed by Amikacin but was sensitive to imipenem. The *Citrobacter* spp. (n=2) only sensitive to all except cotrimoxazole. *Providencia* spp. showed resistance only to Cotrimoxazole and Cefotaxime but was sensitive to the others. Urinary samples which yielded *Enterobacterales* (*E. coli*, *Klebsiella* spp. and *Enterobacter* spp.) showed complete resistance to norfloxacin but were sensitive to nitrofurantoin except for *Klebsiella* spp. which were resistant to nitrofurantoin also (Table 1).

Enterobacter*ales* isolates which were resistant to first line drugs were tested for sensitivity to second line drugs. Of the second line drugs the *E. coli* showed complete resistance to ceftriaxone, netilmicin and cefipime but was sensitive to aztreonam and tetracycline. The *Enterobacter* spp, *Klebsiella* spp., *Citrobacter* spp. showed total resistance to tetracycline, aztreonam, ceftriaxone and cefipime. *Enterobacter* spp. showed sensitivity to netilmicin followed by tigecycline. *Klebsiella* spp showed only minimal sensitivity to tigecycline. *Citrobacter* spp. was sensitive only to netilmicin all others were resistant (Table 2).

Overall amongst 34 *Enterobacterales* isolates the most sensitive was fluoroquinolones (82.3%) followed by imipenem (52.9%), cotrimoxazole (38.2%) and cefipime (29.4). All isolates were resistant to ampicillin. Least sensitive were piperacillin and tazobactam (14.7%)and cefotaxime (8.8%) (Table 1). The 12 isolates showed resistance to first line drugs and among the second line drugs tigecycline was the most sensitive (54%), tetracycline was the least sensitive (6%) (Table 2). Since the *Providencia spp.* are intrinsically resistant to colistin and tigecycline same outcome was established in this study.[10]

Nonfermentative gram negative bacteria (NFGNB) (n=36)

The *Acinetobacter* spp. (n=31) showed resistance to ceftazidime followed by piperacillin-tazobactam, imepenem, ciprofloxacin. It was sensitive to gentamicin and amikacin. *Pseudomonas* spp. (n=4) showed complete resistance to minocycline and 75% resistance to ceftazidime. Most sensitive drug was gentamicin followed by imipenem, piperacillin-tazobactam, ciprofloxacin and amikacin in descending order. *Stenotrophomonas* spp. was sensitive all the first line drugs except imipenem (Table 3).

The *Acinetobacter* spp. (n=11) which were resistant to the first line drugs were tested for second line drugs among which it was sensitive to tigecycline followed by netilmicin and meropenem. It was totally resistant to cefuroxime and tetracycline. *Pseudomonas* was resistant to all the second line drugs (Table 4).

Overall among the nonfermenters (86%) were resistant to ceftazidime, followed by minocycline (69%) and piperacillin and tazobactam (69%). The nonfermenters were most sensitive to aminoglycosides (41%), followed by imipenem (36.1%) and ciprofloxacin (36.1%).

Nonfermentative gram negative bacilli showed resistance to 1st line of drugs, 2nd line drugs were tested for and among the 2nd line like netilmicin, meropenem and tigecycline (10% each) very minimal sensitivity, whereas complete resistance to cefuroxime, aztreonam and tetracycline was observed (Table 4).

Resistance patterns

Klebsiella spp. and *Enterobacter* spp. predominantly ESBL producers whereas *E. coli* showed increased MBL production. All *Citrobacter* spp. showed production of both ESBL and MBL and 1 isolate even showed colistin resistance. *Acinetobacter* spp showed increased production of MBL compared to ESBL, 13 isolates showed production of both ESBL and MBL. In *Pseudomonas* spp the ESBL and MBL was seen in equal proportion (Table 5).

ESBL was produced by a total of 35.7% of the isolates of which 41% by *Enterobacteriaceae* and 30% were by nonfermenters (Figure 3 and Table 5) MBL were 44.2% of isolates 41.1% were by *Enterobacteriacea* family and 47.2% were by nonfermenters (Figure 3 and Table 5).

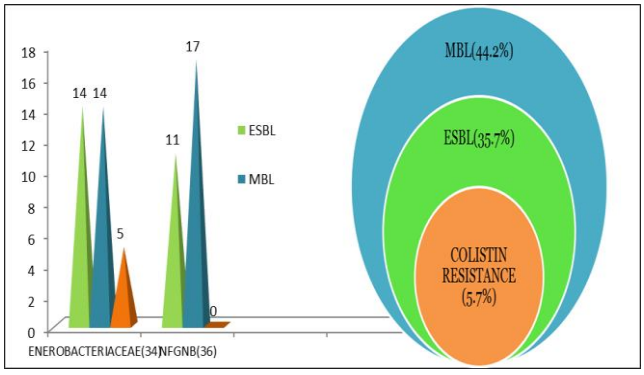

Figure 3: Overall resistance pattern among ICU isolates.

Bacterial isolates that showed resistance to colistin at a concentration higher than 2 µg/ml classified as resistant. Colistin resistance seen in 4 isolates (5.7%) *Enterobacteriaceae* family showed maximum in addition to colistin resistance (5.7%), whereas among nonfermenters there was no colistin resistance at all (Table 6). However 1 isolate was *Providencia* spp. and since there is intrinsic resistance to colistin and tigecycline it was not included for colistin resistance in this study.

Of the above colistin resistant isolates all were extended spectrum beta lactamase producers except for one *Klebsiella* app. isolated from Surgical Site Infection (SSI). The *Providencia* spp. As well as the *Citrobacter* spp. were also MBL producers. However among the above isolates except for the MBL producers all were sensitive to the imipenem. Fluoroqinolones were also active against the above isolates except for the *Enterobacter* spp as well as the *Klebsiella* spp. (Table 1).

Table 1: First line drug resistance pattern in *Enterobacterales*.

Enterobacterales (n=34) (%)	Amp (%)	Ce (%)	I (%)	Cot (%)	G (%)	PT (%)	Cf (%)	Ak (%)	Nit, n (%)	Nx, n (%)
***E. coli*, (n=7)**	100	71	28	57	57	28.5	71	42.8	2 (50)	2, 100
***Enterobacter* spp., (n=9)**	100	100	77	77	100	44	77	44.4	1 (50)	1 (100)
***Klebsiella* spp., (n=15)**	100	66	33.3	60	60	77	80	53	1 (100)	1 (100)
***Citrobacter* spp., (n=2)**	100	50	50	100	50	50	50	50	0	0
***Providencia* spp., (n=1)**	100	100	0	100	0	0	0	0	0	0

% of resistant isolates (Amp-Ampicilin, Ce-Cefotaxime, I-Imipenem, Cot-Cotrimoxazole, G-Gentamicin, Pt Piperacillin/Tazobactam, Cf-Ciprofloxacin, Ak-Amikacin, Nit-Nitrofurantoin, Nx-Norfloxacin

Table 2: Second line resistance pattern in *Enterobacterales*.

Enterobacterales	Net (%)	Te (%)	Tgc (%)	Ao (%)	Cpm (%)
***E. coli*, (n=2)**	100	50	0	50	100
***Enterobacter* spp., (n=5)**	80	100	60	100	100
***Klebsiella* spp., (n=4)**	100	100	50	100	100
***Citrobacter* spp., (n=1)**	0	100	100	100	100

% of resistant isolates (Net-Netilmicin, Te-Tetracycline, Tgc-Tigecycline, Ao-Aztreonam, Cpm-Cefipime).

Table 3: First line drug resistance among nonfermentative gram negative bacteria.

Non fermenter, (n=36)	Ca (%)	I (%)	G (%)	PT (%)	CF (%)	AK (%)	Mno (%)
***Acinetobacter* spp., (n=31)**	80	67	58	77	64.5	51	45
***Pseudomonas* spp., (n=4)**	75	50	0	50	50	50	100
***Stenotrophomonas* spp., (n=1)**	0	50	0	0	0	0	0

% of resistant isolates (Ca-Ceftazidime, I-Imipenem, G-Gentamicin, PT-Piperacillin/Tazobactam, Cf-Ciprofloxacin, Ak-Amikacin, Mno-Minocycline).

Table 4: Second line drug resistance among nonfermentative gram negative bacteria.

Non fermenters, (n=12)	Net (%)	Te (%)	Tgc (%)	Cu (%)	Mrp (%)	Ao (%)
***Acinetobacter* spp., (n=11)**	72	100	90	100	45	100
***Pseudomonas* spp., (n=1)**	100	100	100	100	100	100

% of resistant isolates (Net-Netilmicin, Te-Tetracycline, Tgc-Tigecycline, Cu-Cefuroxime, Mrp-Meropenem.

Table 5: Resistance patterns among ICU isolates.

Organism	ESBL producers	MBL producers	ESBL and MBL producers	Colistin resistance
***E. coli*, (n=7)**	2	3	1	None
***Klebsiella* spp., (n=15)**	5	4	1	2
***Enterobacter* spp., (n=9)**	6	4	2	1
***Citrobacter* spp., (n=2)**	2	2	2	1

Continued.

Organism	ESBL producers	MBL producers	ESBL and MBL producers	Colistin resistance
***Providencia* spp., (n=1)**	1	1	1	Not applicable*
***Acinetobacter* spp., (n=31)**	11	17	5	0
***Pseudomonas* spp., (n=4)**	2	2	1	None
***Stenotrophomonas* spp., (n=1)**	0	1	0	None

* *Providencia* spp. there is intrinsic resistance to colistin and tigecycline

Table 6: Colistin MIC values of resistant organisms.

Organism and sample	No. of isolates	Colistin MIC
***Klebsiella* spp. /SSI (Pus sample)**	2	64 µg/ml
***Citrobacter* spp./SSI (Pus sample)**	1	64 µg/ml
***Enterobacter* spp./ Early onset sepsis (blood sample)**	1	64 µg/ml

DISCUSSION

ICUs are evolving to be an arena of resistant pathogens that constitute a real challenge in terms of treatment as well control. So, in view of acquiring knowledge about antimicrobial resistance patterns for GNB in ICUs from our hospital scenario, this study was done.

In the present study, the majority of the samples that were received from ICU were samples for blood culture (65%) indicating that a common cause for morbidity and mortality in the critically ill patients is sepsis. This result agrees with a previous study by Pien et al which establishes that blood culture (51%) is an essential tool which is used to identify the pathogens and also establish the drug sensitivity so that early and appropriate treatment can be ensued.[13] Appropriate antibiotic therapy itself is starting point for curbing antimicrobial resistance.

In study fluroquinolone resistance was found to be 72% among *Enterobacterales* and 55.5% among non-fermenters which correlates with study by Melinda et al who demonstrated fluoroquinolone resistance of (80-90%) among *Enterobacterales* and (70-75%) among non-fermenters.[24]

In our study multidrug resistant GNB were the most predominant organisms (ESBL-35.7% and MBL-44.2%) isolated among the neonatal and pediatric ICU isolates. Shraddha et al in their study have also observed a rising prevalence of MDR-GNB (60%), specifically ESBL-producing and MBL producing among GNB.[15]

Another study by Mutasim et al revealed that *Acinetobacter* spp. (27.2%), *P. aeruginosa* (23.8%), and *K. pneumoniae* (18.6%) are the most common GNB associated with ICU infections in their hospital. Similarly in this study also *Acinetobacter* spp. (44.7%) were the most common isolate with MDR pattern followed by *Klebsiella* spp. (20%).[9]

In our study *Klebsiella* spp. were more predominant in medical 66%) and surgical (38%) ICU which corresponds to recent review (2020) which also presented *K. pneumoniae* as increasing threat to public health in many Asian countries because of its increasing resistance potential.[16]

To overcome resistance of β-lactamase, it is common practice to administer combination of β-lactam antibiotics and β-lactamase inhibitors to improve their antimicrobial activity. Piperacillin/ tazobactam is one of the most widely used antibiotic compound as empirical therapy in majority of ICU's. In this study resistance to piperacillin/ tazobactam was observed in *Acinetobacter* spp. (77%), *Klebsiella* spp. (77%) Xiao et al in their study established similar >70% resistance to piperacillin/ tazobactam in *Acinetobacter baumanii* and (41-45.9%) resistance by *Enterobacterales* (*E. coli* and *Klebsiella* spp.).[11]

Souli et al in their study have suggested that tigecycline (80-90%) is reliable treatment option for treatment Multidrug resistant *Enterobacterales* and in this study also it have shown significant sensitivity to tigecycline (74%) and hence it can be used in cases of ESBL, MBL producers as reliable resort before choosing colistin.[12]

In our study MBL producers were estimated to be 44.2%, ESBL producers were 35.7% and combined ESBL and MBL producers were 18%, this was observed mainly in *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp., *Acinetobacter* spp., *Pseudomonas* spp. and *Citrobacter* spp. a similar observation was made by Iswarya et al in their study on detection of ESBL and MBL producing gram negative bacilli, (47%) were ESBL producers, 23 (23%) MBL producers and 13 (19%) isolates were both ESBL and MBL producers.[17] Two main mechanisms of carbapenem resistance are acquisition of carbapenemase genes, such as Ambler class A, B, and D beta-lactamases; and decrease in uptake of antibiotics by qualitative or/and quantitative deficiency of porin expression in association with overexpression of beta-lactamases that possess very weak affinity for carbapenemase.[14] Worldwide spread of *Enterobacteriaceae* expressing carbapenemase now represents a significant threat to public health and

requires immediate efforts toward early detection and infection control.[7]

According to the study by Ajlan et al the most frequent carbapenem resistant pathogen was *Klebsiella* spp. (36.1%) followed by *Escherichia coli* (32.9%), *Acinetobacter* spp. (8.4%), *Enterobacter* spp. (5.8%) and *Citrobacter* spp. (3.9%) a similar observation was made in our study (Table 5).[6]

In the present study among GNB colistin resistance was found to be 5.7%, A similar pattern was observed by Kanwalpreet et al in which the prevalence of colistin resistance in GNB was estimated to be 5.6%.[3]

Colistin resistance in MDR GNB was found to be 19.6% in the study by Panigrahi et al but in our study colistin resistance was 5.7% which shows that the prevalence of colistin resistance even though alarming is lesser in our ICU's.[18]

Recently, colistin has been increasingly used as a rescue therapy alone or in combination with one or more other antimicrobials to treat carbapenem-resistant and MDR Gram-negative bacteria.[19] Although colistin currently maintains a high activity level against most *K. pneumoniae* isolates, the decrease in activity against carbapenem-resistant isolates is worrisome.[20] In our study the presence of colistin resistance among *Klebsiella* spp. was 2.8%. Narissa et al in their meta-analysis established that colistin resistance among *Klebsiella* spp. has increased from 4.8-8.2% in a period of 3 years [20]especially among carbapenem resistant strains.

One isolate of *Enterobacter* spp. showed colistin resistance in our study. Colistin resistant *Enterobacter* spp. have emerged in the last decade. A recent study from the British society for antimicrobial chemotherapy (BSAC) resistance surveillance programme revealed that annual colistin resistance rates among *Enterobacter cloacae* complex isolates isolated from 2011-2017 were 4.4-20% and were much higher than those of *Klebsiella* spp. and *Escherichia coli* and there is a proposition that resistance might be due a different mechanism other than the mutations in PmrA/B and PhoP/Q in *Enterobacter* spp.[5]

In our study, 1 isolate of *Citrobacter* spp. showed colistin resistance (MIC 64 µg/ml) it was also ESBL and MBL producer. Similarly, Wand et al demonstrated 16-fold increase in colistin MIC values in their study, genetic analysis revealed that this increased resistance was attributed to mutations in PmrB for *Citrobacter*.[21]The rate of the ESBL production among the *Citrobacter* spp. in this study was comparable to that of other studies by Ali et al.[23] Slow but steady emergence of *Citrobacter* spp. as an uropathogen, resistant to commonly available antibiotics is alarming. Proper surveillance in anti-microbial sensitivity pattern of *Citrobacter* is necessary, and it should no longer be ignored as a commensal.[22]

CONCLUSION

Overall amongst the *Enterobacterales* the most sensitive drug was tigecycline, fluoroquinolones followed by imipenem and cotrimoxazole. Among the nonfermenters most sensitive drugs were aminoglycosides followed by imipenem and ciprofloxacin, netilmicin, meropenem were also senstive. The increased prevalence of MDR gram-negative isolates has shown an immediate need for reassessment of the protocols for antibiotic therapy in ICU. Prolonged hospital ICU admission coupled with unnecessary antibiotic administration increase the spread of MDR pathogens. Therefore, the common risk factors that could be associated with escalating MDR patterns among ICU pathogens should be defined time to time. A high prevalence of ESBL 35.7%, MBL were 44.2 and 5.7% of colistin resistance were present among ICU acquired infections. Hence, timely antibiogram and antibiotic stewardship programs have to be conducted for a better understanding of the type of organism, their sensitivity and resistance pattern, so as to initiate empirical antibiotics in emergency conditions. A routine assessment of local bacterial prevalence and antibiotic susceptibility is mandatory. Equal emphasis has to be given for de-escalation of antibiotics whenever needed. Furthermore, the adoption of "one health" policy by all sectors using antimicrobials is highly essential for completely eliminating the multifaceted problem of emerging antimicrobial resistance.

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