Acquisition of Carbapenem- resistant of gram negative bacilli in critical care units; Molecular Epidemiology

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INTRODUCTION

Infection and antimicrobial resistance are persistent and correlated problems in Intensive care units (ICUs). They are directly or indirectly associated with increased morbidity and mortality. These critical care units have become increasingly important in the past two decades as a hub of hospital acquired infections. ICU-acquired infection rates are found to be five to ten times higher than hospital-acquired infection rates in general ward patients and outbreaks are very common. The acute nature of critically ill patients necessitates the frequent use of broad spectrum antibiotics in confined environments. Among the various risk factors, prior antimicrobial therapy, use of indwelling devices and length of ICU stay are found to be most important. As a result infections with antibiotic-resistant organisms become prevalent in the ICUs and pose a challenge for the clinician dealing with critically ill patients.

The common sites of infection in ICU - acquired infections are the respiratory tract, urinary tract, bloodstream, skin and soft tissue, gastrointestinal tract and cardiovascular system. Among all the pathogens associated with these infections, the incidence of gram-negative bacillary infections has increased more than gram-positives. Aerobic gram negative bacilli of Enterobacteriaceae family e.g. Klebsiella spp., Escherichia coli, Enterobacter spp., Proteus mirabilis and Non-fermenter gram negative bacilli e.g. Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophila, have been most commonly isolated from ICU acquired infections.

The commonly used antimicrobial drugs for gram negative bacilli are beta lactam antibiotics such as third and fourth generation cephalosporin (eg, cefotaxime, ceftriaxone), beta lactam & beta lactamase inhibitor combinations and carbapenems (eg:Imipenem/cilastatin), aminoglycosides(eg, gentamicin, amikacin)Carbapenems being the broadest spectrum are frequently used.

The treatment with beta lactam antibiotics has led to a global spread of drug resistance especially with extended-spectrum Beta-lactamases (ESBLs) which render penicillins, narrow and broad spectrum cephalosporins and monobactams ineffective for treatment. Up to 60% antibiotic resistance rate has been found to cefixime and ceftazidime in ICU settings. In such cases carbapenems are the only available beta lactams for treating life threatening ICU infections potentially caused by MDR bacteria including ESBLs and Amp C enzyme producing Enterobacteriacae, and even for Acinetobacter species. The carbapenems which are presently in clinical use are imipenem-cilastatin, meropenem, ertapenem, doripenem, panipenem-betamipron, and biapenem.

Carbapenem resistance has been reported with increasing frequency worldwide in the last two decades. Production of the enzymes, carbapenemases by gram negative bacilli has been one of the important mechanisms for resistance to carbapenems along with others such as development of efflux pumps, and mutational alterations in the expression and/or function of porins.

Hence the current study was carried out to detect and characterize carbapenemases in gram negative bacterial infections in critical care units by using phenotypic and genotypic methods.

MATERIAL AND METHODS

The present study was a prospective, descriptive type of study conducted in the department of Microbiology of an urban tertiary care teaching hospital extending over a period of 18 months from July 2019 to January 2021. Around 37251 samples for bacterial culture were processed in the laboratory during this period. Out of these 6400 samples were processed from different critical care units(CCU). This number includes 2916 samples from adult intensive care unit(ICU), 1980 samples from medical intensive care unit(MICU), 387 samples from neonatal intensive care unit(NICU) and 1117 samples from pediatric intensive care unit(PICU). Samples included in the study : The specimens of Blood samples(3840), Tracheal secretions(1344), Urine(640), Pus(448), and others which included Tissue, venous Catheter Tips, Sterile Body fluids including CSF, Pleural fluid, Ascetic fluid(768) received from the different CCUs for the bacterial culture and sensitivity during the study period were studied.

***Size of the study sample*:**

All the non-repeat and single clinically significant isolates of gram negative bacilli from various critical care units' samples were included in the study.

Inclusion criteria: Single and non-repeat isolates of gram negative bacilli.

Exclusion criteria: 1.Gram negative bacilli isolates from mixed culture.

2. Clinically non-significant gram negative bacilli isolate.

Processing in laboratory:

The samples were processed for bacterial culture as soon as they were received in the laboratory as per standard microbiology protocol. They were inoculated onto Blood agar (BA), MacConkey Agar (MA). For sputum, blood and body fluids chocolate agar was also inoculated. The inoculated plates were incubated at 37°C for 16-18 hours aerobically.

Any growth on the plates was noted after overnight incubation and if no growth was evident, they were incubated for further 24 hours and discarded thereafter if no growth seen.

Gram-negative bacteria were processed separately and the isolates were identified to the species level and confirmed with standard biochemical tests.

Identification of clinical isolates belonging to various gram negative bacilli species was done in the laboratory as follows:

Colony characteristics on primary isolation:

E.coli: Smooth, glistening, mucoid/non mucoid, convex colonies on blood agar & Lactose Fermenting colonies on MacConkey agar.

K.pneumoniae: Large, dome shaped mucoid, colonies on blood agar & Lactose Fermenting colonies on MacConkey agar.

Other Enterobacteriacae spp. (eg. Enterobacter spp, Citrobacter spp, Proteus spp.) : Smooth, glistening, mucoid/non mucoid, convex colonies, with or without swarming on blood agar & Lactose Fermenting/ Non Lactose Fermenting colonies on MacConkey agar.

Non Enterobacteriacae spp. /Non Fermenters (Acinetobacter spp., Pseudomonas spp. & Others): Smooth/Rough, glistening, mucoid/non mucoid, convex/flat colonies, with/without entire margins, pigmented/non-pigmented, odorless/with odour, with/without hemlolysis on blood agar & Non Lactose Fermenting colonies on MacConkey agar.

Microscopic examination:

Gram stain: Gram negative, straight/curved, non-sporing rods/ coccobacillary forms.

Motility (Hanging Drop Preparation): Motile/Non motile bacilli.

Biochemical Reactions:

The gram negative bacilli of Enterobacteriaceae were identified using the biochemical Reactions mentioned below-

	*TSI	Gas	H_2S	MR	VP	Ind	Cit	PAD	Ure	MOT	Lys	Arg	Orn	ONPG
E-Coli	A/A	+	-	+	-	+	-	-	-	+	+	-/+	+/-	+
K.pneumoniae	A/A	++	-	-	+	-	+	-	+	-	+	-	-	+
E.aerogens	A/A	++	-	-	+	-	+	-	-	+	+	-/+	+	+
C.koseri	Alk/A	+	-	+	-	+	+	-	+/-	+	-	-	+	+
P.vulgaris	Alk/A	-	+	+	-	+	-	+	++	+	-	-	-	-
P.mirabilis	Alk/A	-	+	+	-	-	+	+	++	+	-	-	+	-

*TSI- Triple Sugar Iron Agar; H2S- Hydrogen Sulphide production; MR- Methyl Red test; VP- Voges-Proskauer test; Ind- Indole production; Cit- Citrate utilization; PAD- Phenylalanine Deaminase production; Ure- Urease production; Mot- Motility; Lys,Arg,Orn- Lysine, Arginine, Ornithine Decarboxylation; ONPG- o-Nitrophenyl-ß-D-galactopyranoside test

For identification of **Acinetobacter baumannii** following reactions were employed:

Oxidase	Motility	TSI	Growth at 42° C	glucose	10% Lactose
-	-	Alk/Alk	+	+	+

Pseudomonas was identified by following reactions:

	Oxd	Mot	Pigment	Lact	Manitol	Arg	ONPG	DNAse	Acet	PB300
P.aeruginosa	+	+	+	-	V	+	-	-	+	S
S.maltophila	-	+	-	+	-	-	+	+	-	S
B.cepacia	Weak	+	-	+	+	-	-	-	-	R

Antibiotic Susceptibility Testing (AST):

(By Modified Kirby-Bauer disc-diffusion method):

The AST was carried out as per the CLSI 2015 guidelines

Specimen:18-24 hrs. pure culture of test isolates were used for inoculum preparation for AST.

Quality control:18-24 hrs pure culture of *Escherichia coli* strain(ATCC 25922).

Preparation of inoculum**:** Broth was incubated at 37^0C for two hours or till a suspension of moderate turbidity was obtained. It was then matched to 0.5 Mc Farland standard turbidity giving a bacterial cell density of approximately 1.5×10^8 CFU/ml. The desired turbidity was obtained by diluting with normal saline if required.

Interpretation:

After overnight incubation, the zone diameters (including the 6mm disc) were measured witharulerontheundersurfaceofthepetridishandinterpretedassensitive, intermediate sensitive and resistant according to CLSI 2015guidelines.

ESBL Detection

As per the CLSI 2015 guidelines the ESBL detection tests are recommended for *K.pneumoniae,E.coli and*P.mirabilis.

Screening test for ESBL detection:

A lawn culture of a 0.5 McFarland's suspension of test isolates were inoculated on Mueller Hinton agar plate. Ceftazidime (30ug), Ceftriaxone (30ug), Cefotaxime (30ug), Aztreonam (30ug), discs were applied and incubated overnight at 37^0C in ambient air16-18 hours Zone diameters ≤ 22 mm for Ceftazidime, ≤ 25 mm for Ceftriaxone ≤ 27 mm for Cefotaxime and ≤ 27 mm for Aztreonam indicated ESBL production.

Quality control: *Escherichia coli* ATTCC 25922 were used as negative control.

Confirmatory test for ESBL detection:[320]

Ceftazidime / Clavulanic acid combination disc diffusion test

This was done according to CLSI 2015 guidelines by using a disc of Ceftazidime (30 µg) alone and Ceftazidime +Clavulanic acid (30/10µg). These discs were placed on a lawn culture of the test isolate on a Mueller-Hinton Agar plate at least 20 mm apart from each other and incubated overnight at 37^0C in ambient air for 16-18 hours

Quality control: *E.coli* ATCC 25922 was used as negative control.

The difference in zone diameters with and without clavulanic acid was measured. 5 mm or more increase in diameter of zone of inhibition for Ceftazidime+Clavulanic acid disc compared with diameter of zone of inhibition around Ceftazidime alone was indicative of ESBL production.

Screening test for Carbapenemases- Modified Hodge Test(MHT):

McFarland standard suspension (using direct colony suspension) of *E.coli* ATCC 25922(the indicator organism) in peptonebrothwaspreparedanddiluted1:10inbroth.It wasinoculatedonaMuellerHintonAgarplateasfortheroutinediscdiffusionprocedure. Plate was allowed to dry for 3 to 10minutes. Ertapenem (10mcg) disc was placed in the centre of the plate. Using a 10-µL loop or swab, 3 to 5 colonies of test isolate were picked up and inoculated in a straight line out from the edge of the disc. The streak was at least 20 to 25 mm in length

Positive control for MHT: Klebsiella pneumoniae ATCC 1705

Interpretation: -MHT Positive test showed a clover leaf-like indentation of the *Escherichia coli* ATCC (25922) growing along the test organism growth streak within the disc diffusion zone. MHT Negative test showed no growth of the *Escherichia coli* ATCC (25922) along the test organism growth streak within the disc diffusion.

Test for detection of Class A Carbapenemases:

All the study isolates were subjected to detection of class A using Boronic acid synergy test.

Double disc synergy test (Inhibitor Based Method) by Pournaras (2010) et al

Template for Double Disc Synergy Test

A lawn culture of a 0.5 McFarland's suspension of test isolates were inoculated on Mueller Hinton agar. Ertapenem (10mcg) was placed in the centre and three discs including boronic acid (400mcg), cloxacillin (500mcg) and ceftazidime-clavulanic acid (30/10mcg) were placed around ertapenem disc as shown in figure 4. The distance between ertapenem and other three discs was kept 1.5cm centre to centre.
Boronic acid (400mcg) discs were prepared by pouring on plain discs (6mm), a 4µl of freshly prepared stock solution containing phenylboronic acid 1mg in 1mL of dimethyl sulfoxide.
Plates incubated at 37°C in ambient air for 18-24 hours. Positive Control: K.pneumoniae ATCC 1705
Interpretation:
Positive synergy between ertapenem (10mcg) and boronic acid (400mcg), no synergy between ertapenem (10mcg) and cloxacillin (500mcg) along with positive Modified Hodge

Test and Negative MBL combined disc detection test was considered as KPC-type carbapenemases (Class A). Synergy between ertapenem (10mcg) and cloxacillin (500mcg) was noted because of hyperproduction of AmpC combined with impermeability.
Negative(or weak positive) synergy between ertapenem (10mcg) and ceftazidime-clavulanic acid (30/10mcg) along with positive Modified Hodge Test and Negative MBL combined disc

Molecular methods for detection of carbapenemases gene

Extraction of the Genomic DNA

Extraction of whole genomic DNA from the isolates was carried out using HiPurA™ Bacterial Genomic DNA Purification Spin Kit (MB505 HI Media Laboratories Pvt. Ltd.) as perthemanufacturer'sinstructions.Allthereagentsexceptforethanolwerepartofthekit content.

Procedure

1. 24-48 hours old culture of bacterial isolates were taken
2. Around 7-10 colonies of the isolate were inoculated into BHI broth (M210) and incubated at 37°C for 18 – 24hrs.
3. 1.5mLofbacterialbrothwaspelletedbycentrifugation.Thepelletwasresuspendedin alkaline lysis solution and 20µL of Proteinase K followed by incubation at 55°C for30 mins in a drybath.
4. RNase A solution was added to the sample and incubated at room temperature for 5 mins with further addition of 200µL Lysis solution. The mixture was agitated in vortex mixture thoroughly for few seconds and incubated at 55°C for 10 mins in a drybath.
5. After incubation, 200µL of ethanol (95 – 100%) was then added and mixed and the lysate solution was transferred onto the spin column and centrifuged.
6. Thespincolumnwasfurtherwashedbycentrifugationwith500µLofPreWashBuffer and Wash Buffer solutions respectively. A dry spin was carried out to ensure the removal of ethanol.
7. Thespincolumnwasthenplacedinaclean1.5mLmicro centrifugetubeand200µLof DNA was eluted in Elution buffer. The extracted whole genomic bacterial DNA was stored in −20°C freezer until further processing.

8. DNA yield from all the extracted samples was determined on a Nano Drop Spectrophotometer (Nano Drop ND-1000**,** Thermo Scientific) by measuring the absorption at 260 nm and purity was calculated based on 260/280ratio.

Polymerase Chain Reaction(PCR):

All the isolates were subjected to PCR for detection of following genes:

1. KPC
2. NDM-1
3. VIM
4. OXA-48

The PCR assays were carried out using Takara PCR 118 Thermal Cycler Dice (TP600 System) [Takara Bio Inc., Japan]. The primers for the PCR assays were obtained from a commercial source(Eurofins,Bangalore,India)(Table1).HI Media's PCR reagents were used for the study. These included 10mM deoxynucleotide triphosphates (dNTPs) mix (MBT078), 5U Taq DNA polymerase (MBT060A) supplied with 10X PCR Buffer and 25mM $MgCl_2$ and Molecular Biology Grade Water [MBGW](ML024).The experiments were carried out to standardize and optimize the PCR assay for characterization of carbapenemases encoding genes. The assay was standardized as uniplex PCRs for KPC-type,NDM-1, and VIM and OXA-48 gene targets

Table 1: Primer Sequences

Primer	Primer sequence (5'-3')	Amplicon size (bp)
KPC-F	ATGTCACTGTATCGCCGTCT	893
KPC-R	TTTTCAGAGCCTTACTGCCC	
KPC-F	GGCAGTCGGAGACAAAACC	177
KPC-R	CCCTCGAGCGCGAGTCTA	
KPC-F	CATTCAAGGGCTTTCTTGCTGC	538
KPC-R	ACGACGGCATAGTCATTTGC	
NDM1-F	GGGCAGTCGCTTCCAACGGT	475
NDM1-R	GTAGTGCTCAGTGTCGGGAT	
VIM-F	TTTGCTCGCATATCGCAACG	500
VIM-R	CCATTCAGCCAGATCGGCAT	
OXA-48-F	GCTTGATCGCCCTCGATT	281
OXA-48-R	GATTTGCTCCGTGGCCGAAA	

The PCR mix composition (20µL) for all the four genes is as follows:

Table 2: PCR mix composition for KPC, NDM-1 and OXA-48

Component	**Working Stock**	**Final concentration**	**Total volume (µL)**
$MgCl_2$ (mm)	25	2.0	1.6
dNTPs (mM)	10	0.8	1.6
Taq Polymerase (U)	5	1	0.2
Buffer(X)	10	1	2
Primer Forward(pmol/ µL)	10	0.5	1.0
Primer Reverse (pmol/µL)	10	0.5	1.0
Nuclease free water (µL)	-	-	10.6
DNA template (µL)	-	-	2

The control strains used for the PCR assays used are:

Strain name	**ATCC number**	**Resistant gene**
K. pneumoniae	BAA 1705	KPC
K. pneumoniae	BAA 2146	NDM

Agarose gel electrophoresis

The PCR products for the respective PCRs were electrophoresed in a 1.5% Low electro endosmosis (EEO) agarose (RM273) containing 0.6µL of EtBr using 50X Tris Acetate EDTA (TAE) buffer (ML016) diluted to 1X TAE and visualized under ultra violet (UV) light using Gel Doc system 50bp ladder (MBT084) was used for identifying the banding pattern in respective gels.

RESULTS

A total o f6400 samples from CCUs were processed during the study period of 18months from July 2019 to January 2021. From 181 specimens different gram negative bacilli were isolatedasasinglesignificantpathogen.Outofthe181gramnegativebacilli,57(31.5%) were identified as *Acinetobacter baumannii*, 49(27%) were confirmed as *Klebsiella pneumoniae*, 18(10%) were confirmed as *Pseudomonas aeruguinosa*, 21(11.6%) were identified as *Escherichia coli*, 11 were *Acinetobacter-calcoaceticus baumannii* complex, 11(6.1%) were *Citrobacter koseri*, 9(5%) were *Enterobacter aerogens*, 2(1.1%) were *Proteus mirabilis* and one 1(0.5%) each were *Proteus vulgaris*, *Burkholderia cepacia* and *Stenotrophomonas maltophila*

Correlation of MHT results with Carbapenemases detection by molecular methods

Bacterial isolates	MHT	Number	KPC	NDM-1	VIM	OXA-48	Total
K.pneumoniae	+	**30**	-	**20**	**8**	**13**	41**
	-	3	-	1	-	-	1
E.coli	+	**8**	-	**5**	-	**1**	6
C.koseri	+	**6**	-	**3**	**1**	**1**	5
E.aerogens	+	**7**	-	**6**	**1**	**5**	12**
A.baumannii	+	**14**	-	**4**	**2**	**3**	9
	-	9	-	-	4	2	6
***Acinetobacter* spp.**	+	**2**	-	**1**	-	-	1
P.aeruginosa	+	**3**	-	-	-	**2**	2
	-	1	-	-	-	1	1
Total	+	70	-	39	12	25	**76****
	-	13*	-	1	-	3	**4**

**Only those MHT negative isolates which were positive with screening tests for carbapenemase production were subjected to molecular methods*

***Includes the isolates detected with multiple carbapenemases*

Beta lactam antibiotic sensitivity profile :*E.coli*(n=21) and *C.koseri* isolates(n=11)

Antibiotic Class	*E.coli*			*C.koseri*		
	Sensitivity Profile			Sensitivity Profile		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Penicillin						
Piperacillin	5(23.8%)	4(19%)	12(57.1%)	0(0%)	0(0%)	11(100%)
Beta lactam/lactamase inhibitor combination						
Piperacillin-Tazobactam	8(38.1%)	0(0%)	13(61.9%)	0(0%)	1(9.1%)	10(90.9%)
Ceftriaxone+ Sulbactam+EDTA	21(100%)	0(0%)	0(0%)	0(0%)	4(36.4%)	7(63.6%)
Cephaloporins						
Cephamycins						
Cefoxitin	7(33.3%)	4(19%)	10(47.6%)	0(0%)	0(0%)	11(100%)
3rd generation cephaloporins						
Ceftriaxone	0(0%)	1(4.8%)	20(95.2%)	0(0%)	0(0%)	11(100%)
Ceftazidime	2(9.5%)	2(9.5%)	17(81%)	0(0%)	0(0%)	11(100%)
Cefoperazone	2(9.5%)	1(4.8%)	18(85.7%)	0(0%)	0(0%)	11(100%)
Cefotaxime	0(0%)	0(0%)	21(100%)	0(0%)	0(0%)	11(100%)
4th generation cephalosporins						
Cefepime	8(38.1%)	3(14.3%)	10(47.6%)	0(0%)	0(0%)	11(100%)
Monobactams						
Aztreonam	0(0%)	0(0%)	21(100%)	0(0%)	1(9.1%)	10(90.9%)
Carbapenems						
Imipenem	13(61.9%)	4(19%)	4(19%)	3(27.3%)	1(9.1%)	7(63.6%)
Meropenem	13(61.9%)	3(14.3%)	5(23.8%)	0(0%)	2(18.2%)	9(81.8%)
Ertapenem	13(61.9%)	3(14.3%)	5(23.8%)	1(9.1%)	3(27.3%)	7(63.6%)

.Beta lactam antibiotic sensitivity profile: *E.aerogens*(n=09) and *Proteus* spp. isolates (n=03)

Antibiotic Class	*E.aerogens*			*Proteus* spp.		
	Sensitivity Profile			Sensitivity Profile		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Penicillin						
Piperacillin	0 (0 %)	0 (0 %)	9 (100 %)	0(0%)	0(0%)	3(100%)
Beta lactam/lactamase inhibitor combination						
Piperacillin-Tazobactam	0 (0 %)	0 (0 %)	9 (100 %)	0(0%)	0(0%)	3(100%)
Ceftriaxone+ Sulbactam+EDTA	0 (0 %)	4 (44.4 %)	5 (55.6 %)	0(0%)	1(33.3%)	2(66.7%)
Cephaloporins						
Cephamycins						
Cefoxitin	0 (0 %)	0 (0 %)	9 (100 %)	0(0%)	0(0%)	3(100%)
3rd generation cephaloporins						
Ceftriaxone	0 (0 %)	0 (0 %)	9 (100 %)	0(0%)	0(0%)	3(100%)
Ceftazidime	0 (0 %)	0 (0 %)	9 (100 %)	0(0%)	0(0%)	3(100%)

Cefoperazone	0 (0 %)	1 (11.1 %)	8 (88.9 %)	0(0%)	0(0%)	3(100%)
Cefotaxime	0 (0 %)	0 (0 %)	9 (100 %)	0(0%)	0(0%)	3(100%)
4th generation cephalosporins						
Cefepime	0 (0 %)	0 (0 %)	9 (100 %)	1(33.3%)	2(66.7%)	0(0%)
Monobactams						
Aztreonam	0 (0 %)	0 (0 %)	9 (100 %)	0(0%)	1 (33.3%)	2(66.7%)
Carbapenems						
Imipenem	0 (0 %)	1 (11.1 %)	8 (88.9 %)	3(100%)	0(0%)	0(0%)
Meropenem	0 (0 %)	1 (11.1 %)	8 (88.9 %)	3(100%)	0(0%)	0(0%)
Ertapenem	0 (0 %)	0 (0 %)	9 (100 %)	3(100%)	0(0%)	0(0%)

Beta lactam antibiotic sensitivity profile: *A.baumannii* (n=57)

Antibiotic Class	**Sensitivity Profile**		
	Sensitive	Intermediate	Resistant
Penicillin			
Piperacillin	13(22.8%)	2(3.5%)	42(73.7%)
Beta lactam/lactamase inhibitor combination			
Piperacillin-Tazobactam	13(22.8%)	6(10.5%)	38(66.7%)
Ceftriaxone+ Sulbactam+EDTA	0(0%)	25(43.9%)	32(56.1%)
Cephaloporins			
Cephamycins			
Cefoxitin	5(8.8%)	2(3.5%)	50(87.7%)
3rd generation cephaloporins			
Ceftriaxone	9(15.8%)	1(1.8%)	47(82.5%)
Ceftazidime	7(12.3%)	0(0%)	50(87.7%)
Cefoperazone	5(8.8%)	3(5.3%)	49(86%)
Cefotaxime	16(28.1%)	0(0%)	41(71.9%)
4th generation cephalosporins			
Cefepime	0(0%)	11(19.3%)	46(80.7%)
Monobactams			
Aztreonam	8(14%)	13(22.8%)	36(63.2%)
Carbapenems			
Imipenem	16(28.1%)	7(12.3%)	34(59.6%)
Meropenem	17(29.8%)	3(5.3%)	37(64.9%)

Sensitivity profile of *K.pneumoniae* to other class of antimicrobial agents

(Zone diameter breakpoint interpretation as per CLSI 2015 guidelines)

Antibiotic Class	Sensitivity Profile		
	Sensitive	Intermediate	Resistant
Aminoglycosides			
Gentamycin	15(30.6%)	1(2%)	33(67.3%)
Tobramycin	14(28.6%)	3(6.1%)	32(65.3%)
Amikacin	17(34.7%)	1(2%)	31(63.3%)
Netilmycin	14(28.6%)	0(0%)	35(71.4%)
Tetracyclins			
Tetracycline	34(69.4%)	0(0%)	15(30.6%)
Fluoro-quinolones			
Ciprofloxacin	11(22.4%)	6(12.2%)	32(65.3%)
Ofloxacin	13(26.5%)	9(18.4%)	27(55.1%)
Folate pathway inhibitors			
Cotrimoxazole	13(26.5%)	1(2%)	35(71.4%)
Phenicols			
Chloramphenicol	26(53.1%)	6(12.2%)	12(24.5%)
Lipopeptides			
Polymyxin B	49(100%)	0(0%)	0(0%)
Glycylcyclines			
Tigecycline	49(100%)	0(0%)	0(0%)

Distribution of Carbapenemases in CCUs

	Number	ESBL	AmpC	KPC	MBL	Class D
CCU	65	24	2	3	6	16
MICU	62	14	3	6	4	18
PICU	53	8	12	7	14	13
NICU	1	0	0	0	0	0
Total	181	46	17	16	24	47

CONCLUSION

Klebsiella pneumoniae and *Acinetobacter baumannii* were the predominant GNBs causing infections in CCUs.Carbapenemases production was found to be an important mechanism of carbapenem resistance as indicated by positive Modified Hodge Test. Very high prevalence of NDM-1 and OXA-48 seen in the present study indicates dissemination of these genes in Enterobacteriaceae. The commonly employed primers for molecular detection of KPC type were not able to detect phenotypically screened class- A-isolates as none of them showed positive result in this study.

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