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Original Research Article

ANTIBIOTIC SUSCEPTIBILITY AND TREATMENT OPTIONS AMONG CARBAPENEM RESISTANT ENTEROBACTERIACEAE

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Abstract

Background: Antibiotic resistance is among the top tier list posing threat to human health and the rate of Carbapenem resistance enterobacteriaceae is concerning as it limits therapeutic options for treatment. Rationale use of the available antibiotics will prevent emergence of resistant bacterial infections. The aim of this study was to determine the antibiotic-resistant pattern of Carbapenem non- susceptible enterobacteriaceae. **Materials and Methods:** This is a cross-sectional study conducted in Index medical college and hospital, Indore. The determination of Antibiotic sensitivity testing for carbapenem resistant enterobacteriaceae by Kirby bauer disk diffusion method was done and these strains were further tested for carbapenamase production. **Result:** The occurance of carbapenem non-susceptible enterobacteriaceae was 24.8% which was isolated from various samples. Colistin showed high sensitivity for CRE followed by tigecycline. Out of 145 cultures positive CRE, 97 were carbapenemase positive. Among 97 Carbapenemase positive 81 were OXA- 48, 22 were NDM and 4 were both. **Conclusion:** Clinicians to adhere to stringent antimicrobial treatment policies and healthcare workers should adhere to infection control practices. Further studies should focus on combination therapy of CRE.

INTRODUCTION

Enterobacteriaceae are common cause of infections like urinary tract infections, meningitis, pneumonia, blood stream infections etc and are also a part of normal flora of the intestine8. These organisms develop antibiotic resistance and a global concern from past few decades. 9 Multidrug resistant infections caused by enterobacteriaceae are treated by Carbapenems.[1] Carbapenems are beta-lactam antibiotics considered as last choice of drug used in treating these infections and also the reason for increase in rate of MDR bacteria due to its frequent use. The emergence of carbapenem non-susceptible enterobacteriaceae is a rapidly growing public health problem because of its ability to be resistant to most used antimicrobials.[2] Several mechanisms render to carbapenem resistance: porin loss, production of carbapenemase enzymes, efflux pumps and beta lactamase production 10 The need of the hour is to work towads development of newer antibiotics and develop strategies to treat these CRE's. Early methods of phenotypic detection of CRE are necessary in patients who show high resistance to an organism. Carbapenem resistance lead to serious challenges for the physicians pushing them to use colistin despite having nephrotoxic effect.[2-7] Colistin resistance is also reported widely due to frequent use of this drug against CRE.[8-14]

This study was conducted to evaluate the resistance pattern of CRE to various antibiotics currently available and to promote help in developing new regimens to treat these infections. To administer early therapy there is a need of rapid detection and susceptibility profile.

MATERIALS AND METHODS

Study Setting

This study was conducted in Department of Microbiology at a tertiary care hospital in Indore. Carbapenem resistant enterobacteriaceae will be differentiated from carbapenem susceptible enterobacteriaeae and further analysed by biochemical tests and antibiotic panel. The study was carried out from December 2021 to January 2023.

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Methodology

Various samples received at our laboratory-like blood, urine, sputum, body fluids, tips, swabs and pus were processed further. The CRE was isolated based on clinical culture which showed growth of Carabapenem resistant organism of the family Enterobacteriaceae from a patient sample and proceed with phenotypic identification followed with exclusion of duplicate samples.

Bacterial Identification

Identification of gram negative bacilli was done by gram stain, culture on blood and macconkey agar kept at 37ºC for incubation and after growth was visible gram stain, oxidase and catalase test, biochemical testing done (IMViC panel including Triple sugar iron agar, urease test, Nitrate and Oxidation – fermentation test). Antibiotic susceptiilty testing performed on Mueller hinton agar by Kirby bauer disk diffusion method.

Following incubation, the zone of inhibition around 10µg Meropenem and 10 µg Imipenem disc was measured according to CLSI 2021, the strain was further subjected to CARBA- NP and mCIM test for presence of carbapenemases.

Antibiotic Testing

The strains of CRE were used to evaluate susceptibility of CRE. The various antibiotics used for susceptibility test were Ampicillin, Gentamicin, Tobramycin, Ampicillin/ Sulbactam, Cefuroxime, Piperacillin-tazoactam, Ciprofloxacin, Amikacin, Tigecycline, Amoxicillin-clavulunate, Tetracycline, Aztreonam done by Kirby bauer disc diffusion method for all samples. All the above antibiotics including Nitrofurantoin and Fosfomycin for urine samples by Kirby bauer disc diffusion method and MIC for colistin by BMD method.

Statistics

For categorical variables percentage was calculated. Analysis done using Excel and SPSS.

Phenotypic testing of CRE –

mCIM method:

Take 1µl Loopful of the identified bacteria from overnight incubated blood agar plate and emulsify in 2ml tryptone soy broth. Immersion of 10 µg of Meropenem disk into the broth. Incubate for four hours at 35ºC. An MHA plate is inoculated with E.coli 25922 comparing the turbidity of control strain with 0.5 Mcfarland . Allow the plate to dry for 3-10 minutes. Remove the Meropenem disk from the suspension and place it on an MHA plate. Incubate the MHA plate at 35ºC for 18-24 hours . Then interpret according to CLSI 2021.

CarbaNP Test

Two microcentrifuge tubes are taken and labeled. 100 µl of extraction reagent (Mtris HCL buffer) is taken in each tube. Loopful (1µl) of bacteria is emulsified in both the tubes. A different control tube with only extraction reagent is kept without the organism in it. 100µl of solution A is added to first tube and solution B is added to the other tube. Vortexing is done. Incubate it for 2 hours at 35º. The test if gives positive before 2 hours are reported as carbapenmase producers.

Genotypic test done after extraction by Hi-PCR Carbapenemase gene multiplex probe kit.

RESULTS

Among the 583 enterobacteriaceae isolated one fourth of bacteria were Carbapenem resistant enterobacteriaceae. Antibiotic sensitivity testing was done for all carbapenem resistant isolates. The frequency of causative organisms isolated are Klebsiella pneumonia (n= 69) 47.5%, Escherichia coli (n=28.2%), Citrobacter freundii (n= 23) 15.8% and Proteus spp. (n=12) 8.2%.

The antimicrobial resistance rates of all isolates were high against beta- lactams (>89%). Furthermore, all CRE strains showed complete resistance to ampicllin , amoxyclav, ceftazidime and cefuroxime. High resistance rates seen towards tobramycin, piperacillin/ tazobactam, ciprofloxacin, aztreonam, gentamicin. Most strains of Carbapenem resistant Enterobacteriaceae were sensitive for colistin, Tigecycline and Amikacin. Colistin (85.5%) and Tigecycline (63.4%) showed highest sensitivity among all antibiotics. Amikacin and Tetracycline could be used as alternatives for treatment as CRE showed sensitivity to them.

All strains of Klebsiella except 10 isolates were resistant to colisitin by BMD method. CRE isolated from urine samples (n=64) showed high susceptibility to Nitrofuratoin 80%, Fosfomycin 87%.

Table 1: Antibiotic Resistance profile of CRE isolated.

Antibiotics	Klebsiella pneumoniae (n=69)	Escherichia coli (n=41)	Citrobacter freundii (n=22)	Proteus spp. (n=12)
AMP	69(100)	41(100)	22(100)	12(100)
AMC	69(100)	41(100)	22(100)	12(100)
GEN	65(94.2)	37(90.2)	21(95.4)	11(91.6)
TOB	52(75.3)	32(82.9)	16(72.7)	10(83.3)
CAZ	69(100)	41(100)	22(100)	12(100)
PTZ	54(78.2%)	33(80.4%)	18(81.8%)	10(83.3)
A/S	69(100%)	41(100%)	22(100%)	12(100)
CXM	69(100)	41(100)	22(100)	12(100)
CIP	54(78.2%)	34(82.9%)	18(81.8%)	10(83.3)
AK	32(46.3%)	23(56%)	14(63.6%)	7(58.3)
TIG	21(30.4%)	12(29.2%)	8(36.3%)	11(91.6)
TE	42(60.8%)	29(70.3%)	9(40.9%)	7(58.3)

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AT	56(81.1%)	30(73.1%)	17(77.2%)	9(75)
CL	10(14.4)	0(0)	0(0)	12(100)

Table 2: NIT and FO resistant pattern for CRE from urine isolates

Isolates (n=64)	Nitrofurantoin	Fosfomycin
Escherichia coli(n=39)	12(30.7)	8(20.5)
Klebsiella (n=23)	6(26)	5(21.7)
Citrobacter freundii (n=2)	1(50)	0(0)
Proteus spp(n=0)	-	-

A lot has changed in the data of CRE in past few years. This study has shown increasing resistance which was low in previous studies.8

Among the isolates most common gene identified was OXA-48 (51.7%) followed by NDM (11%). Combination of NDM and OXA-48 gene was seen in 2.4 % isolates. Most common resistant gene isolated overall was OXA- 48 (55.8%)

DISCUSSION

Antibiotic resistance is on rise globally and CRE is among the current challenge the clinicians are facing because of lack in development of new antibiotics. Colisitin use has tremendously risen in the past decade despite it having nephrotoxic effect. Many studies around the world have shown increase in hospital acquired infections caused by gram negative resistance bacteria.[2] Our study has focused on monitoring the emergence of CRE in our tertiary setting and characterize the type of carbapenamase that is produced to know the current scenario.[15-19]

In the present study there was 100% resistance shown to Ampicillin, Amoxyclav, Cefuroxime and Ceftazidime by all CREs isolates. Resistance of these organisms to the current available antibiotics has tremendously increased over a span of few years. It may be due to frequent used of high generation antibiotics for treatment purposes.[20-23]

Our study shows the highest sensitivity of Colistin 85.5% and 3rd generation cephalosporin show 100% resistance. A study showed that many carbapenemase producers are susceptible in vitro to the glycylcycline group (Tigecycline), but there is rapid increase in resistance to this drug during treatment. Morrill et al, reported monotherapy is not effective against infection caused by carbapenem-resistant bacteria.[2] In this study we found 35.8% tigecycline resistant CRE.[24-26]

Most common bacteria isolated among CRE were Klebsiella pneumoniae and Escherichia coli. Comparison of these two organisms, the prevalence was 47% of Klebsiella pneumoniae and 28% for Escherichia coli. The prevalence rate of CRE 24.8% in this study.[27,28]

This study showed uropathogenic CRE isolates that were resistant to carbapenems were sensitive to nitrofurantoin 70.3% and Fosfomycin was 79%. In another study done by Banerjee S et al. 95% uropathogenic isolates were sensitive to fosfomycin.[11] Pokharel K et al. found that the CRE isolated in their study were susceptible to Nitrofurantoin.[8] Some studies show fosfomycin a choice of treatment for CRE isolates from urine samples.[2] In our study the resistant rate of CRE to fosfomycin is 20.9%.[29-31]

Colistin is considered among the last line of antibiotic for treating CRE.2 In our study we found 14.4% colistin resistance to Klebsiella pneumonia by BMD method compared to few other studies that showed colistin resistance of 20-30 % among CR Enterobacterales. All other isolates were found susceptible to colistin. Study done by Bir R et al. showed E.coli 16% reisistance to colistin followed by K. pneumonia 14.5%.[15]

Polymicrobial infection with pseudomonas aeruginosa was isolated from three pus samples that also isolated Carbapenem resistant Klebsiella pneumonia from two samples and carbapenem resistant citrobacter freundii from one sample. The infections caused by CRE got more severe when a polymicrobial infection was found and high dose of antimicrobials were used during treatment.[32,33]

Giri el al. included 50 CRE samples in their study from Maharashtra west region, India concluded detection of 90 % NDM gene , 60% OXA -48 and 12% VIM gene 7 and a similar study on 624 CR gram negative isolates showed Klebsiella pneumonia (59.9%) the most common organism isolated in which the predominant gene was NDM 33.6% followed by OXA-48(32.6%) and NDM.[10] In this study, Most common OXA-48 recognized in Klebsiella pneumonia followed by NDM and combined OXA 48 + NDM.[34,35]

CONCLUSION

Clinicians should avoid using broad spectrum antibiotics for experimental treatment. Infection control practices and use of antibiotic policy need to be improved. Our study showed colistin resistance in ten isolates, Colistin used as the last line drug for treating CRE and these patients should be treated by combination therapy.

Limitation: Proteus spp are intrinsically resistant to certain antimicrobials so no other new options were tested for CR proteus spp. Studies should be done on Carbapenem resistant proteus species as there is limited data available.

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