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RESEARCH ARTICLE

DETECTION OF FREQUENCY OF BACTERIA CARBAPENEMASE PRODUCING IN PEDIATRIC CASES IN TERTIARY CARE HOSPITAL

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Abstract

Introduction:New strains of bacterial species are now showing multidrug resistance & causing nosocomial and community-acquired infections. The rapid rise in the prevalence and clinical consequences of infection caused by bacteria that produce carbapenemase is a global health concern in pediatric cases.

Material And Method: This study was done in bacteriology laboratory of PCMS & RC, from to February 22 to February 23. Total 268 samples of pediatric patients were included in the study. Identification of bacteria was done by conventional methods. Primary screening of Carbapenemase producing bacteria was done by Kirby-Bauer disk diffusion method by using meropenem (10ug) &Imipenem (10ug) as per as CLSI guideline. Confirmation of Carbapenemase producing bacteria was done by 3 different methods using Boronic acid test, Imipenem-EDTA synergy test & Combination disc test method.

Result: In our study the frequency of carbapenemase producing bacteria were found to be 52 % in pediatric cases. Imipenem-EDTA synergy test & Combination disc test method were found to be more effective in detecting carbapenemase producing bacteria into Boronic acid test.

Conclusions: The present study demonstrates the presence of high level of Multidrug resistant Among carbapenemase producing GNB isolated from pediatric cases as assessed in 2023 in central India.We conclude that the prevalence of phenotypically confirmed carbapenemase producer amongGram negative bacteria is quite useful.The comparison of multiple phenotypic assays for the detection of carbapenemaseproducing bacteria indicates that the Combination disc test and Imipenem-EDTA test provides the highest rate of positivity.

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Introduction:-

New strains of bacterial species are now showing multidrug resistance and causing nosocomial and community -acquired infections, particularly Gram - negative bacteria, which are the leading cause of infections in the urinary tract, bloodstream, and lower respiratory tract[1] The rapid rise in the prevalence and clinical consequences of infection caused by bacteria that produce carbapenemase is a global health concern in pediatric cases.[2] Carbapenemase -producing bacteria, also known as Carbapenem Resistant Enterobacteriaceae (CRE), have developed resistant to nearly all existing medicines, making CRE infections extremely difficult to treat and sometimes lethal.[3] First The Carbapenemaseidentified in Enterobacteriaceae was the chromosomally encoded NmcA from an Enterobacteriaceae clinical isolate in 1993.[4] Carbapenems have traditionally been used to treat infections caused by Escherichia coli and Klebsiella pneumoniae that produce extended -spectrum -lactamases, as well as carbapenemase.Rising reports of infections in the United States and around the world, combined with an increasingly medically complicated pediatric population, imply that CRE could become a major nosocomial pathogen in pediatric hospitals in the coming years.[5]

Material And Method:-

This cross-sectional study was conducted in the Department of Microbiology, Peoples College of Medical Sciences & Research Centre. A total of 268 Of under 12 year pediatric patients attending the (IPD/OPD) department were included in the study. The duration of this study was 1 year from February 2022 to January 2023.

Specimen collection;

The various samples, such as pus, sputum, urine, and endotracheal exudates,csf etc. were collected aseptically in a clean, dry, wide mouth crew cap leak proof sterile container in aseptic condition .and blood sample were collected on blood culture bottle .and all sample immediately send to the micro lab in case of delay [6.7.11]

Identification Of Pathogens

The pathogens were identified by standard microbiological techniques by studying their colony characteristics, morphology, motility and biochemical reactions.[8.11.12]

Primary screening of cabarpenemase producing Bacteria

All the isolated of GNB were screened for carbapenem resistantby using Meropenem (10 ug) or Ertepenem (10 ug) disc . CLSI guidelines were followed for interpreting the result a zone size of <22 mm for ertapenem or< 23 mm for Meropenem was considered to be Carbapenem resistant. [9.10.11]

Antimicrobial susceptibility test

Antimicrobial susceptibility testing (AST) was performed by Kirby-Bauer disc diffusion method as per the CLSI guidelines.[9.10.11]

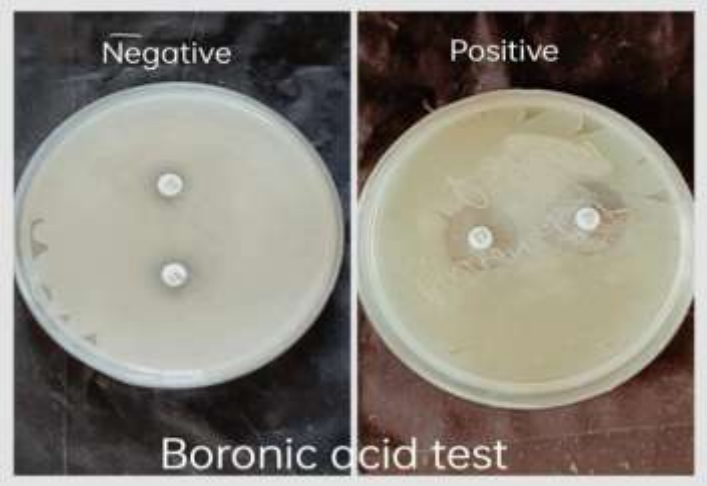
PhenotypicMethod

1.Boronic acid test[13]

Klebsiella PneumoniaeCarbapenemase (KPC) and class A and C beta -lactamases are both inhibited by phenylboronic acid. Forthe phenotypic detection of Klebsiella Pneumoniae Carbapenemase - producers, the boronic acid test has been proposed.

Procedure:-

1. Make a McFarland bacte rial suspension (0.5 McFarland).
2. Usea sterile cotton swab to inoculate and two meropenem discs to put.
3. On one of the two meropenem discs, pour 20 ul of phenyl boronic acid (20 g/L).
4. Incubate for 18 -24 hours at 37C.



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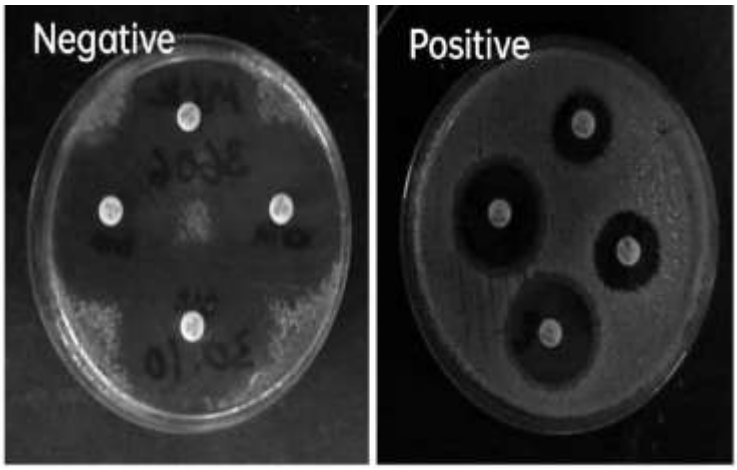
Combination meropenem disc test[13]

This test combines the EDTA and the boronic acid tests Onto a single plate, and it was developed in Greece in response to the development of Gram negativeisolates Klebsiella Pneumoniae Carbapenemase and metallo -beta -lactamases . The test distinguishes between bacteria that are carbapenem -susceptible, bacteria that produce Klebsiella Pneumoniae Carbapenemase , bacteria that produce metallo -beta -lactamases , and bacteria that produce double - carbapenemase .

Procedure

1. Make a McFarland bacterial suspension (0.5 McFarland).
2. Use a sterile cotton swab to inoculate and two meropenem discs to put.
3. On the 2nd disc, pour 10 l Ethylene -Diamine -Tetraacetic Acid 0.1 M and 20 ul phenylboronic acid. 20 g/L on the 3rd disc, 20u l phenylboronic acid 20 g/L on the 4th disc, and 10ul Ethylene - Diamine -Tetraacetic Acid 0.1 M on the 5th disc.
4. Incubate for 18 -24 hours at 37C. Interpretation The inhibition zones of the four meropenem discs



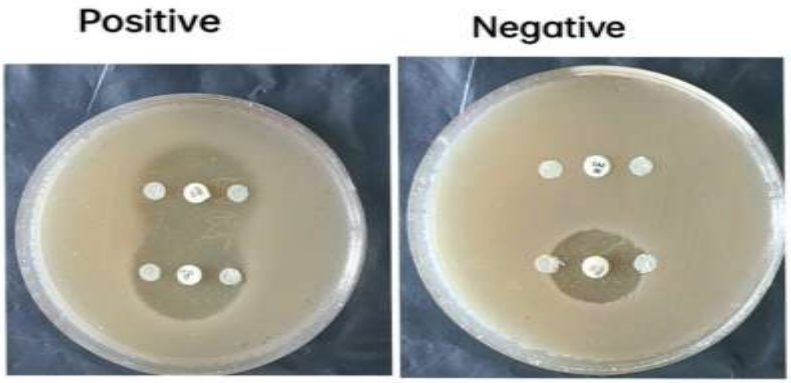
Imipenem-EDTA synergy test[13]

Imipenem - Ethylene -diamine -tetra acetic acid synergy test: The polyamino carboxylicacid EDTA binds metal ions like zinc and can inactivate metallo -beta -lactamases. As a result, it's employed to detect metallo -beta -lactamases production in clinical isolates phenotypically.

Procedure

1. In a 0.1 M EDTA solution, soak paper discs.
2. Make a McFarland bacterial suspension(0.5 McFarland)
3. Place an imipenem and ceftazidime disc in the centre of the plate and inoculatewith a sterile cotton swab.

4. In relation to the antibiotics, place the EDTA discs on both sides.
5. Incubate for 18 -24 hours at 37C.

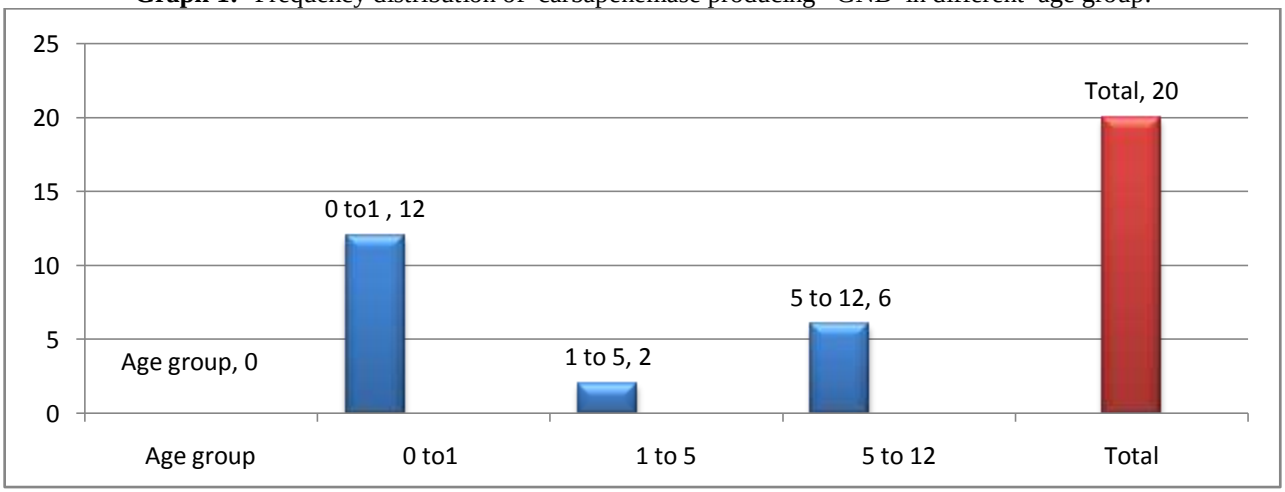


Imipenem -EDTA synergy test

Results:-

Age Distribution

Graph 1:- Frequency distribution of carbapenemase producing GNB in different age group.



In the present study a total of 38 isolates the frequency of GNB (Entrobacteraceace and Non fermeters) were characterized and is shown in Table The maximum number of isolates were E coli14 (36.84%) followed by Klebsiella pneumoniae 7 (18.42%) and Pseudomonas aeruginosa 6 (15.78%),

Table 1:- Frequency distribution of carbapenemase producing bacteria.

S. NO	Isolated Organism	Frequency of Total clinically isolated	Percentage of clinically isolated bacteria
1	Escherichia coli	13	34.21%
2	Klebsiella pneumonia	7	18.4 %
3	Pseudomonas aeruginosa	6	15.78%
4	Acinetobacter baumannii	4	10.52%
5	Entrococcus faecalis	3	7.89%
6	Citrobacter koseri	2	5.26%
7	Proteus mirabilis	1	2.63 %
8	Salmonella typhi	2	5.26 %
	Total	38	100 %

Baesd on Primary screening and phenotypic test

ll the 28 isolates which were tested positive by screening test&were subjected to carbapenem production by phenotypic method & were confirmed by boronic acid test. Imipenem –EDTA synergy test,andcombination meropenem disc test as shown in figure 3A total of 20 out of 28 iso lates (71 %) tested positive of synergy test production by by boronic acid test,imipenem –EDTA and combination meropenem disc test.

Table3:- Primary screening for detection of cabropenemaseproducing bacteria in GN Based Antimicrobial susceptibility.

S. NO	Isolated Organism	No of Isolated in Primary screening of carbapenem	FrequencyofCarbapenemase Producing bacteria	Boronic acid test	Imipenem –EDTA synergy test	Combination dics test
1	Escherichia coli	7	3 (20%)	1	3	3
2	Klebsiella pneumoniae	6	5 (25%)	4	5	5
3	Pseudomonas aeruginosa	5	4 (20%)	3	4	4
4	Acinetobacter baumannii	4	3 (10%)	2	3	3
5	Entrococcus faecalis	2	2 (10%)	1	2	2
6	Salmonella typhi	2	1 (5%)	2	1	1
7	Citrobacter koseri	1	1 (5%)	1	1	1
8	Proteus mirabilis	1	1 (5%)	1	1	1
	Total	28	20(100)%	N=16	N=20	N=20

Table 4:- Antimicrobial susceptibility pattern of Carbapenemase producing bacteria.

S.NO.	Antimicrobial agent	Number of(%)resistant isolate	Number of(%)Intermediate isolate	Number(Of(%)sensitive isolate
1	Cefixime	21(55%)	1 (2.90%)	16 (42 10%)
2	Cefotaxime	17 (44%)	4 (11.27%)	17(44.73%)
3	Meropenem	21(55%)	0(0%)	17 (45%)
4	Imipenem	18(47%)	2(6%)	18 (47%)
5	Cefepime	15 (42%)	3 (8%)	19 (50%)
6	Ertapenem	15 (42%)	1 (2.11%)	22(55.89)

Discussion:-

The emergence ofcarbapenemase- producing Entrobacteriaceae has become a mojar concern to pediatricinfection with relatively few therapeutic options, as they are responsible for the both health care-associated and community acquired infections. Gram negative bacteria specially members of the family Entrobacteriaceae are the most commonly implicatedoraganism of the multidrug resistant. Carbapenem are the drug of the choice and in fact last option left to deal with the MDR organism.

In the present study,the frequency of GNB (Entrobacteraceace and Non fermeters) is38 out of the 258 in total number of sample. the maximum number of isolated E coli.14 (36.84%) followed by Klebsiella pneumoniae 7 (18.42%) and Pseudomonas aeruginosa 6 (15.78%), Acinetobacter baumannii 4(10.52%) Entrococcus species 3(7.89%), Salmonella typhi

2 (5.26%),Citrobacterkoseri 1 (2.63%) Proteus mirabilis 1 (2.63%). The similar study conduct by Rendani et al (2017)[13] to Among 39 bacterial isolates, the most frequently isolated bacterial species was Acinetobacter baumannii (n= 10), followed by Klebsiella pneumoniae (n= 7), Escherichia coli (n= 5), Enterobacter cloacae (n= 5), and Stenotrophomonas maltophilia (n= 4).

A plethora of phenotypic test is available for the detection of carbapenemase –producing bacteria test is organism with a varying sensitivity and specificity. The most recommended is boronic acid 58 for detection of Klebsiella producing carbapenemase(KPCs) and imipenem –EDTA test for detection of MBL –producer.Boronic acid compounds have been shown to be excellent AmpC inhibitors [13] Subsequently, they have beenshown to be excellent KPC inhibitors [24]. In thepresent study, we evaluated the use of boronic acid disktests for the phenotypic detection of OXA-48- andNDM-producing GNB isolates in the clinical laboratory.The inhibitory activity of APB (300 and 600 μg) with IMP and meropenem as antibiotic substrates is tested against a collection of clinical isolates. The clinical isolates included carbapenemase producers 20 (24 OXA-48 producers and eight NDM producers) and 18 carbapenemase non-producers. For The result are detected by boronic acid are is 15 out of 20 are carbapenemase producing bacteria..this study similar to conduct by RashaElsherif et al .2016 [14]The clinical isolates included carbapenemase producers 23 (24 OXA-48 producers and eight NDM producers) and 15 carbapenemase non-producers.

The antibiotic susceptibility out of 38 isolated tested. The highest percentage of resistance were recorded in cefixime (55%)the resistant rate is also high Meropenem(55%) and Resistant rate of imipenem (47%) the resistant rate of cefotaxine (44 %). the resistant rate for Cefepime and Ertapenem were (42 %) for both. The highly resistantantimicrobial susceptibility is detected in Klebsiella pneumoniae or Entrococcus faecalis .and highly sensitive pattern of deleted in Escherichia coli. (22.2%), this study compares to other one study conduct by Tsegaye Alemayehu et al. [2022][15] .to the rates of the multidrug, extensive, pan drug were (86.5), (43.3), and 1.8, respectively. followed by cefuroxime 52 (91.2%), cefotaxime 94 (88.7%), ceftazidime 40 (83.3%), ciprofloxacin 47 with high level of resistance. meropenem 13 (11.7%).

Conclusion:-

The present study demonstrates the presence of high level of Multi drug resistant among carbapenemase producing GNB isolated from pediatric cases as assessed in 2023 in central India.We conclude that the prevalence of phenotypically confirmed carbapenemase producer amongGram negative bacteria isquite useful .The Comparison of multiple phenotypic assays for the detection of carbapenemaseproducing bacteria indicates that the Combination disc test and Imipenem-EDTA test provides the highest rate of positivity. We also recommend to perform antimicrobial susceptibility testing in routine to detect Carbapenemase producing bacteria as it can aid in the treatment of patients

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