Section: Microbiology

Original Research Article

MOLECULAR CHARACTERIZATION OF THE CARBAPENEM RESISTANT ESCHERICHIA COLI ISOLATES AT A TERTIARY CARE HOSPITAL IN INDORE, INDIA

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ABSTRACT

Background: The development and spread of carbapenem resistance in Escherichia coli is currently a major health problem around the globe. Current study mainly investigates the genotypic detection of carbapenem resistant Escherichia coli isolates after screening as a carbapenem resistant.

Materials and Methods: This study was carried out in the department of Microbiology, Index Medical College, and Hospital & Research Centre (IMCHRC) Indore (M.P.). Different clinical sample were collected from the patients attending IPD & OPD of Medical College, Hospital & Research Centre (IMCHRC) Indore (M.P.). Total 96 E.coli isolates was examined regarding carbapenem resistant genes according to different clinical sample, wards and age group.

Results: In our study, NDM genes were higher in E. coli isolates 88(91.66%), NDM+OXA-48 gene was found 8 (8.33%). The majority NDM were detected from 31-40 years of the age group.

Conclusion: The rate of carbapenem resistant Escherichia coli isolates is spreading very fast and it’s becoming most important health problem as the parts of infectious disease. Therefore, in order to stop the spread of resistant bacteria and change treatment approaches, it is crucial to identify the shifting pattern of resistance as soon as feasible.

Keywords: Escherichia coli, New Delhi Metllo beta Lectamase, Polymerase Chain Reaction, Extended Spectrum of Beta Lectamase.

INTRODUCTION

A member of the Enterobacteriaceae family, Escherichia coli is a facultative anaerobic, enteric, Gram-negative bacterium. Humans and other warm-blooded species' digestive tracts naturally contain Escherichia coli.[1]Escherichia coli, a very ubiquitous commensal of both adults' as well as children’s gastrointestinal tracts, are frequently employed in research on the prevalence of antibiotic resistance in commensal bacteria.[2]It has been well acknowledged in recent years that the normal flora plays a latent role in the development and dissemination of infections' resistance to antibiotics.[3]Research on common flora in underdeveloped nations has revealed resistance rates to a variety of antimicrobial treatments.[4]2.2 million people in developing nations pass away each year from infections linked to diarrhea, and 130–175 million people worldwide get simple UTIs each year, with Escherichia coli responsible for more than 80% of these cases.[5-7] E. coli, which spreads from the gastrointestinal tract to extra-intestinal locations such the bloodstream, urinary tract, and central nervous system, is one of the main causes of infectious illnesses.[8,9]E. coli is a leading cause of sickness and mortality worldwide. The treatment of E. coli infections is challenging because of the rise in antibiotic resistance. In several environments, E.

coli species are becoming increasingly resistant to frequently given antibiotics.[10] numerous factors contribute to the rise in antibiotic resistance in microorganisms. Common bacterial resistance is caused by a number of causes, including the hazardous use of antibiotics, incorrect dosage, and insufficient therapy in both humans and some animals. Multidrug-resistant (MDR) bacterial infections are increasingly prevalent in both the community and hospital settings.[11]The frequency of bacterial isolates exhibiting the ESBL phenotype varies by geographic location, with low prevalence rates of 3–8% recorded in Japan, Sweden, and Singapore contrasted with significantly greater prevalence rates identified in various investigations.[12]One of the main mechanisms of resistance shown in E. coli is the synthesis of extended spectrum β-lactamase enzymes (ESBL). Because they are resistant to all cephalosporin's and penicillin's, the bacteria that produce ESBL are very dangerous. The two most common forms of these ESBLs are TEM (40–72%) and CTX-M (60–79%).[13,14]The most extensively distributed enzyme among the various CTX-M type ESBLs in the globe is CTX-M-15. It was initially discovered in an isolate from India in 1999, and as a result, it spread over the world.[15]E. coli carbapenemase resistance is especially significant because these drugs are frequently the last resort for successful treatment. With prevalence rates of 38–92 and 15–26 percent, respectively, New Delhi metallo-β-lactamase (NDM) and carbapeneme-hydrolyzing oxacillinase-48 (OXA48-like) are the most frequently documented carbapenemases in E. coli,[16]in our nation, a growing public health concern is the rise in antibiotic resistance among Gram-negative organisms, such as E. coli. This suggests that ongoing AMR monitoring is necessary to record any shifting patterns in our geographic area.

MATERIALS AND METHODS

Study Design: Prospective and observational study.
Study Setting: Department of Microbiology at a tertiary care hospital in Indore, India.
Study Period: December 2020 to December 2022
Ethical Consideration: Before the commencement of the study, clearance from the institutional ethics committee (IEC) was taken (IEC approval letter No: MU/Research/EC/Ph.D./2020/57). The study subjects were explained in detail the purpose of the study and were assured confidentiality of their identity. Written informed consent was taken from all the patients before collecting their samples.
Study Population: All patients admitted in the hospital wards and ICUs or visiting the outpatient department of the hospital.
Sampling: All consecutive, non-duplicate samples were included till the sample size was met.
Inclusion Criteria: Isolates of *Escherichia coli* that was resistant to either ertapenem or meropenem or both. The breakpoint for determining resistance was equal or less than 18 mm and 19 mm for ertapenem (10 µg) and meropenem (10 µg), respectively.
Exclusion Criteria: Isolates of *Escherichia coli* that were intermediate or susceptible to ertapenem and meropenem, and other Gram negative bacteria.
Methodology: Clinical samples such as urine, pus, sputum, endotracheal aspirate (ETA), bronchoalveolar lavage (BAL), and blood were collected aseptically as per the standard operating procedure (SOP). They were aseptically inoculated onto Blood and MacConkey agar plates and incubated at 37^{0}C for 16-18 hours. *Escherichia coli* isolates were identified based on their culture characteristics and conventional biochemical testing. Patients of all age groups were included in the study. The isolates that were resistant to either meropenem or ertapenem or both as per CLSI M100 2020,[17] standards by the Kirby-Bauer disk diffusion method were included in the study. After the screening of the resistant Later on, selected *E. coli* isolates, were transferred to the Molecular laboratory at Department of Microbiology, where their DNA was extracted and amplified to detect for the presence of Carbapenemase genes; *bla*NDM, *bla*KPC, *bla*IMP, *bla*VIM, blaOXA-23, blaOXA-48, blaOXA-51, blaOXA-58 by RT-PCR test.

Isolation of DNA

Cells for harvest: Use a recombinant *Escherichia coli* culture that has been cultivated in a medium with the right antibiotic for at least 14–16 hours. In 2.0 ml micro centrifuge tube with a lid, add the required quantity for the culture. Centrifuge the cells for 1 minute at 13,000 rpm. Delete the culture medium that was supernatant. Once the bacterial pellet has been resuspended, mix it thoroughly with 250 µl of Resuspension Solution (HP1) (DS0020) using a gentle pipette until no visible cell clumps remain. To lyses the cells, add 250 µl of Lysis Solution (HP2) (DS0021). Neutralization Solution (HN3) (DS0022) in the amount of 350 µl should be mixed completely right away by 4-6 gentle turns of the tube. The sample should be centrifuged at 13,000 rpm for 10 minutes to create a compact white pellet. The supernatant should be carefully removed before the sample is transferred to a HiElute Miniprep Spin Column (Capped) and centrifuged for one minute at 13,000 rpm. Discard a liquid that is flowing through.The column should be cleaned with 500 µl of Wash Solution (HPB) (DS0032), and the mixture should then be centrifuged for one minute at 13,000 rpm. Add 700 µl of diluted Wash Solution (HPE) (DS0024) to the column to be washed, and centrifuge at 13,000 rpm for 1 minute.A clean 2.0 ml uncapped collection tube should be used for the column, and 50 µl of either the Molecular Biology Grade Water (ML064) or Elution Buffer (ET) (DS0040) should be added. Centrifuge it for 1 minute at 13,000 rpm after letting it stand at room temperature (15 to 25°C) for 1 minute. 1µl of extracted DNA was used for PCR amplification.

Amplification data

Sample	"Ct value"	
	Positive control	Negative control
Positive control for NDM	19.5	-
Positive control for KPC	21.4	-
Positive control for IMP	18.7	-
Positive control for VIM	18.6	-
IC	23.17	23.15

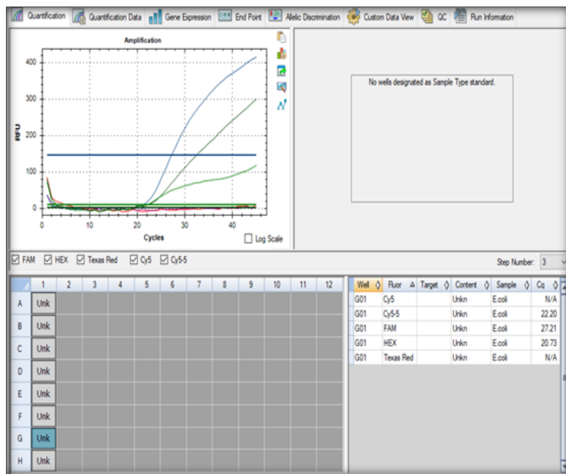
Using HiMedia's Hi-PCR® carbapenemase gene (multiplex) probe PCR kit, an amplification plot of NDM, KPC, IMP, and VIM genes with Ct values is shown in the image. The sample types have a significant impact on the outcomes.

Sample	"Ct value"	
	Positive control	Negative control
Positive control for OXA-23	14.21	-
Positive control for OXA-48	14.89	-
Positive control for OXA-51	16.39	-
Positive control for OXA-58	15.93	-
IC	19.42	19.49

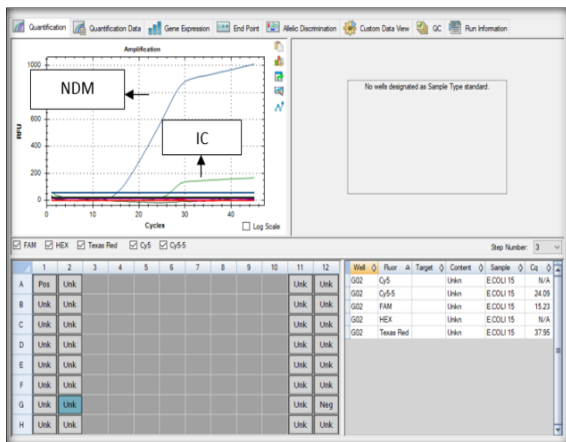
Using HiMedia's Hi-PCR® carbapenemase gene (multiplex) probe PCR kit, an amplification plot of OXA-21, OXA-48, OXA-58, and OXA-23 genes with Ct values is shown in the image. The sample types have a significant impact on the outcomes.

Interpretation

"Ct value"	Finding
≤40	Positive
>41 /N/A	Negative



Cycle 2: Positive for NDM and OXA 48



Cycle 1: Positive for NDM

RESULTS

A total of 428 Escherichia coli were isolated during the study period. 96 isolates of the total 428 isolates were found to be carbapenem resistant. The prevalence rate of carbapenem resistant Escherichia coli was estimated to be 22.42%. [Table 1].As per the distribution of NDM, OXA-23, OXA-48, and OXA-51 genes in E. coli isolates. In our study, NDM genes were higher in E. coli isolates 88(91.66%), NDM+OXA-48 gene was found 8 (8.33%) where as other gene like OXA-23 and OXA-51 was absent. [Table 2]. Show the distribution of isolates based on presence of NDM, OXA-23, OXA-48, OXA-51 gene in different age groups. In E. coli, NDM gene was present in 23 isolates in age group 31-40 years, 18 in age group 21-30 years, 18 in age group 41-50 years, 14 in age group 51-60 years, 05 in age group 61-70 years, 5 in age group 71-80 years, 4 in age group 81-90 years and 01 isolate in age group <20 years. NDM+ OXA-48 genes were present in 3 isolates in age group 51-60 years and 01 in age groups 21-30, 31-40, 41-50, 61-70 and 71-80 years of the age. [Table 3]. According to gender wise distribution of Escherichia coli isolates. In males, NDM genes were present in 45 (90 %) isolates and NDM+OXA-48 were present in 5 (10%) out of the 50 isolates. In females, NDM genes were present in 43 (93.47%) isolates and NDM+OXA-48 were present in 3 (6.52%) out of 46 isolates. All these differences were not statistically significant between gender and genes in the Escherichia coli isolates. ($p > 0.05$). [Table 4]. Show the distribution of genes in different sample types in

case of E. coli isolates. In our observations, NDM gene was present in 15 blood samples, in 10 pus samples, in 2 sputum samples and in 62 urine samples. NDM+OXA-48 genes were present in 4 blood samples, in 1 in pus sample, and in 3 urine samples. Other genes were not detected in any sample types. [Table 5] As per the genes distribution in E. coli isolates in various wards. In our observations, NDM gene was found in 4 isolates from CCU, 5 from emergency ward, 14 from gynecology ward, 15 from ICU, 25 from medicine ward, 2 from NICU, 2 from PICU, 15 from surgery ward, 1 from tuberculosis ward and 5 from SICU. NDM+OXA-48 were found in 2 isolates from ICU and medicine ward, 1 fromNICU, PICU, surgery ward and tuberculosis ward.

Table 1: Isolates distribution based on presence of NDM, OXA-23, OXA-48, OXA-51 gene in E. coli isolates

Name of Gene	*E. Coli*	
	No of isolates	Percentage (%)
NDM	88	91.66%
NDM, OXA-23	0	0.00%
NDM, OXA-23, OXA-48, OXA-51	0	0.00%
NDM, OXA-48	8	8.33%
NDM, OXA-51	0	0.00%

Table 2: Distribution of different genes in different age group of E. coli

AGE GROUP	NDM, OXA-23, OXA-48, OXA-51	NDM	NDM, OXA-23	NDM, OXA-48	NDM, OXA-51
<20 years	0	1	0	0	0
21-30 years	0	18	0	1	0
31-40 years	0	23	0	1	0
41-50 years	0	18	0	1	0
51-60 years	0	14	0	3	0
61-70 years	0	5	0	1	0
71-80 years	0	5	0	1	0
81-90 years	0	4	0	0	0
>90 years	0	0	0	0	0

Table 3: E. coli Isolates distribution based on presence of NDM, OXA-23, OXA-48, OXA-51 gene.

Name of genes	*Escherichia coli* isolates					
	In Male (50)	Percentage	In Female (46)	Percentage	Chi - square	p- value
NDM	45	90%	43	93.47%		
NDM, OXA-23	0	0.00%	0	0.00%		
NDM, OXA-23, OXA-48, OXA-51	0	0.00%	0	0.00%		
NDM, OXA-48	5	10.00%	3	6.52%		
NDM, OXA-51	0	0.00%	0	0.00%	0.38	0.53
No significant Association						

Table 4: Distribution of genes based on sample type in E. coli isolates

Name of genes	Sample Type					
	E. TIP	BAL	BLOOD	PUS	SPUTUM	URINE
NDM	0	0	15	10	2	62
NDM, OXA-23	0	0	0	0	0	0
NDM, OXA-23, OXA-48, OXA-51	0	0	0	0	0	0
NDM, OXA-48	0	0	4	1	0	3
NDM, OXA-51	0	0	0	0	0	0

Table 5: Distribution of genes based on ward type in E. coli isolates

Name of Gene	Ward Type										
	CCU	Dialysis	EMG	Gynae	ICU	Medicine	NICU	PICU	Surgery	TB	SICU
NDM	4	0	5	14	15	25	2	2	15	1	5
NDM, OXA-23	0	0	0	0	0	0	0	0	0	0	0
NDM, OXA-23, OXA-48, OXA-51	0	0	0	0	0	0	0	0	0	0	0
NDM, OXA-48	0	0	0	0	2	2	1	1	1	1	0
NDM, OXA-51	0	0	0	0	0	0	0	0	0	0	0

DISCUSSIONS

Among Enterobacteriaceae members, the Escherichia coli isolates are the most common causative organisms to cause both hospital and community acquired infections. The MDR strains of Enterobacteriaceae causes very severe illnesses that are challenging to treat with available antibiotics. Due to treatment failure by available antibiotics it leads to cause high mortality in patients suffering from these infections. The carbapenem are

considered as antibiotics as a last resort for the above MDR infections. Carbapenem is safe and very effective treatment use for severe infection caused by Enterobacteriaceae. But unfortunately, the last decade is said to be the decade of quickly spreading carbapenem resistance Enterobacteriaceae worldwide. Mechanism to carbapenem antibiotics resistance is either due to production of enzyme carbapenemase (which destroys the beta-lactam ring of carbapenem antibiotic) or by porin loss (which leads to decreased permeability of bacterial cell wall). The most common mechanism is through production of enzyme carbapenemase. The carbapenemase Enterobacteriaceae (CRE) are isolated from both nosocomial as well community-acquired infections; however, nosocomial spread seems to be the main mode of transmission. We have done Genotypic tests for different genes responsible for production of carbapenemase (NDM, VIM, IMP, KPC, OXA23, OXA48, OXA51 and OXA58) and compared with results of various researchers across the globe which is given in the table below: -

We identified the various genes of carbapenemase production by PCR. In our study, we sought the genes KPC, VIM, IMP, NDM, OXA-23, OXA-48, OXA-51, and OXA-58. In E. coli, the NDM gene was found in 91.66% of isolates. Our findings were consistent with the studies of Anandam et al,[18,19] and Han et al. who also found NDM gene in 93.75% and 95.97% of E. coli isolates, respectively. We had NDM+OXA-48 gene in 8% of E. coli isolates. Our study was consistent with the study of Sekar et al,[20] that found the NDM+OXA-48 gene in 6.74% of E. coli isolates, respectively. However, in the study by Giri et al,[21] NDM+OXA-48 gene was found in 45.45% of E. coli isolates. We did not find other genes for E. coli in our study.

We compared the results of our study with various studies. Most of the isolates in our study (23.77%) were from people between the ages of 31 and 40 years, while only 2.5% came from people under 20 years. However, a study by Pawar et al,[22] [169] found that the age range 41–60 years had the highest percentage of isolates (37.05%) while the age group >80 years had the lowest percentage of isolates (4.7%).

Study	Most common organism and most common genes.	
	Escherichia coli.	
	NDM	NDM+OXA-48
Anandametal.	93.75%	
Han et al.	95.97%	
Sekaret al.		6.74%
Giri et al.		45.45%
Present study	**91.66%**	**8%**

CONCLUSION

Carbapenem resistant Escherichia coli isolate is rapidly increasing now a days and it is the most important causes of problem among the infectious disease. Rapidly pattern of resistant need to be detected as soon as possible to prevent the carbapenem resistant Escherichia coli and improve the strategies of the treatment. It is very difficult to choose right antibiotics for treatment of carbapenem resistant strains. There are a number of ways to stop carbapenem resistance from developing in a hospital or healthcare setting, including the use of stringent infection control protocols, prudent antibiotic prescription practices, the establishment of surveillance systems for antibiotic resistance, and antibiotic cycling. Every microbiology laboratory should regularly monitor and document carbapenem resistance.

Recommendation

1. In our study NDM genes were more prevalent in Escherichia coli isolates and only 8 Escherichia coli isolates carried the NDM+OXA 48 geneswhere as other genes like NDM+OXA23 and NDM+OXA 51 was absent.
2. Only eight genes of carbapenemase production were identified due to non- availability of PCR kits having primer for others genes.
3. The identification of other mechanisms or resistance (porin loss etc.) to carbapenems was not performed.

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