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Original Research Article

Carbapenem Resistance in Clinical Isolates of Escherichia Coli among Patients Visiting A Tertiary Care Hospital, in Central India

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Abstract:

Introduction: The emergence and increase of Carbapenem resistance in Escherichia coli is now posing a serious threat to human health around the world. Our study aimed to investigate the phenotypic and genotypic detection of Carbapenem resistance among E. coli isolates.

Material Method: The present study was carried out in the Department of Microbiology, Index Medical College, Hospital & Research Centre (IMCHRC) Indore (M.P.). Various clinical samples were collected from the patients attending Medical College, Hospital & Research Centre (IMCHRC) Indore (M.P.). Total 215 E.coli isolates were examined regarding age, sex, departments and Carbapenemase resistance among different clinical specimens such as urine, pus, blood, CSF and respiratory secretions received in microbiology laboratory.

Results: It is observed that out of 215 E.coli isolates, about 153 (71.16%) isolates were from male patients while 62 (28.83%) were from female patients. The majority (53.95%) of isolates were from urine samples. Out of 215 E. coli isolates, total 63 (29.30%) were carbapenem resistant.

Conclusion: the rate of Carbepenem resistant E.coli is rising very quickly and becoming most important problem in the part of infectious diseases. Hence, now it is very important to detect the changing pattern of resistance as soon as possible to prevent the sread of resistant bacteria and modifying the strategies of treatment.

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

Introduction

Escherichia coli is an enteric, gram-negative, facultative anaerobic bacteria which belongs to the Enterobacteriaceae family. Escherichia coli are a normal flora of intestinal tract of warm blooded animals including humans [1]. Escherichia coli are a near-ubiquitous commensal of the gastrointestinal tract of children and adults has often been used in studies of the incidence of antibiotic resistance in commensal bacteria [2]. In last few years, it has been worldwide recognized that the normal flora has latent role in the emergence and spread of antimicrobial resistance in pathogens [3]. In developing countries, studies on normal flora shown

that studies on normal flora show high resistance rates to diverse antimicrobial agents [4]. Every year 2.2 million people die in the developing countries due to diarrhea associated infection and around the world, 130-175 million patients suffer annually with uncomplicated urinary tract infection (UTI) and >80% of them are because of Escherichia coli.[5-7] One of the major reason of infectious diseases is *E. coli* that extend from the gastrointestinal tract to extra-intestinal sites such as the bloodstream, urinary tract and central nervous system [8,9].

Around the world, *E. coli* represents a major reason of morbidity and mortality. Due to the emergence of antimicrobial resistance the treatment of *E. coli* infections is complicated. *E. coli* species are being ever more resistant to commonly prescribed antibiotics in many settings[10]. Among bacteria, there are a variety of reasons of rising antibiotic resistance. Haphazard utilization of antibiotics, improper dosing, and incomplete treatment in both humans and also some animals are some of the factors leading to development of resistance in common bacteria. Infections of Multidrug resistant (MDR) bacteria are now becoming quite common, not only in hospital settings but also in the community [11].

Across different geographical regions, the prevalence of bacterial isolates expressing the ESBL phenotype varies with low rates of 3 - 8% reported in Japan, Sweden and Singapore compared to much higher prevalence rates recognized in different studies [12]. The production of extended-spectrum β-lactamase enzymes (ESBL) is one of the major mechanisms of resistance observed in *E. coli.* The ESBL-producing strains are of particular concern as these are resistant to all cephalosporins and penicillins. Among these ESBLs, TEM (40-72%) and CTX-M (60-79%) types are more prevalent [13,14]. Across the worldwide, of the different CTX-M type ESBLs, the most widely disseminated enzyme is CTX-M-15. In 1999, it was first identified in an isolate from India and consequently became widespread worldwide [15].

Carbapenemase resistance has particular importance among *E.coli* as these agents are often the last line of effective therapy. Among Carbapenemases, New Delhi metallo-β-lactamase (NDM) and carbapeneme-hydrolysing oxacillinase-48 (OXA48-like) are the most common reported in *E. coli* with prevalence rate of 38-92 and 15-26 percent, respectively[16].

The increasing antimicrobial resistance among Gram–negative organism including E. coli is a growing public health concern in our country. This indicates the need for continuous monitoring of AMR to document any changing trends in our geographical region.

Material and Methods

Study was carried out for the detection of carbapenem resistance in Escherichia coli isolates in the Microbiology department at Index Mdical College, Hospital and Research Centre, Indore (M.P.) in the duration of 2 years from January 2019 to January 2021. Total 215 E.coli isolates were included for the detection of Carbapenem resistance in different types of specimens such as pus or wound swab or aspirate, urine, other body fluids like CSF and respiratory secretions like sputum, bronchoalveolar lavage etc. The study desighn was aaproved by ethical committee and the protocol of study was reviewed and approved by the research cell of Index Medical College, Hospital & Research Centre, Indore (MP.). Ethical Committee also acquired the ethical approval.

Laboratory diagnosis:

Following procedure was followed for the procedure of all the specimens.

Inclusion criteria

- Different specimens with E.coli isolate of patients, who were admitted in ICU and wards. All kind of specimens like

urine, sputum, endotracheal aspirate, bronchoalveolar lavage, blood, pus, wound and respiratory tract specimens, body fluids, high vaginal swab, swab from non healing ulcers and CSF were included in the study.

Exclusion criteria

Clinical samples with polymicrobial growth ≥3 types of organisms.

Sample processing: Blood agar and MacConkey's agar was used to culture all the specimens except blood. Brain heart infusion broth was used to culture and then it was also cultured on both the agar. Cutured plates were placed in incubator at 37˚C for overnight. Organism identification was carried out with Gram's staining, motility test, colony characteristics and at last confirmed with biochemical tests.

Carbepenem resistance detection in E.coli

1. **Disc Diffusion test to detect Imipenem resistance:** CLSI guidelines were followed for the determination of routine antibiotic susceptibility and interpretation. Suspension of isolates of E.coli strains was prepared which was adjusted to turbidity equivalent to 0.5 McFarland standards and then it was inoculated on Mueller Hinton agar. Imipenem and Meropenem discs (10µg) were placed on the surface of the agar and that plate was incubated from overnight at 37˚C. <19 mm zone was considered as Imipenem resistant. For quality control, E.coli ATCC 25922 was used.

2. Imipenem and Imipenem+EDTA combined disc test: E.coli isolates suspension opacity adjusted to 0.5 McFarland opacity standards) was used for inoculation on Mueller Hinton agar. After drying both the discs, Imipenem (10µg) and Imipenem with EDTA (10µg/750µg) were placed with the 20mm distance from centre to centre, then the plate was placed for incubation at 37˚c for overnight. If the zone of inhibition was increased with ≥7mm quality control, E.coli ATCC 25922 was used.

3. Modified Hodge Test: Mueller Hinton agar was used for the inoculation of the suspension of E.coli ATCC 25922 with dilution of 1/10 of 0.5 McFarland turbidity. Allowed to dry for few minutes. In the centre a disc of 10µg Meropenem/ 10µg Ertapenem was placed in the centre and a straight line from edge to edge of test organism was streaked, and then incubated for overnight at 37˚C. then it was observed for a clover leaf type indentation at the intersection of the two organism. Klebsiella pneumonia ATCC BAA-1705 and Klebsiella pneumonia ATCC BAA-1706 were used as positive and negative controls respectively.

4. **MBL detection by Imipenem (4-256µg/ml)/ Imipenem EDTA (1-64 µg/ml) E Strip:** Mueller Hinton agar was used for the inoculation of the orgsnism. A sterile wooden swab was dipped in the standard suspension and this swab was streaked on the entire surface of the plate. Then MIC strip was placed on the centre of the plate with an applicator and then pressed firmly on the cantre of strip. Then the plate incubated for overnight at 37˚C. For quality control E.coli ATCC 25922 was used.

Results

Mostly of the 1050 samples, 20.47% showed the growth of E.coli. Remaining 196 (18.66%) patient's samples showed growth of other organisms. E.coli was Isolated more often from urine samples (53.95%), after that by blood (14.88%) and sputum (9.76%). A detailed description of distribution of E.coli isolates among different clinical samples in the present study is shown in table 1.

Table 1: Distribution of Escherichia coli Isolates from various clinical samples. (n=215)

Specimen	No. of isolates	Percentage %
Urine	116	53.95
Blood	32	14.88
Sputum	21	9.76
Pus	17	7.90
Pleural fluid	11	5.11
CSF	8	3.72
Ascitic fluid	4	1.86
Synovial fluid	4	1.86
BAL fluid	2	0.93
Total	**215**	**100**

Among 215 E.coli isolates, 153 (71.16%) isolates were from male patients while 62 (28.83%) were from female patients which is shown in Table 2.

Table 2: Gender wise distribution of E.coli isolates: (n=215)

Gender	Total No. of Cases	Percentage
Male	153	71.16
Female	62	28.83
Total	**215**	**100**

Of the 215 samples, 61-70 yrs (24.65%) was the age group in which maximum number of E.coli isolates were found followed by (15.34%) between age group of 31-40 yrs and (14.88%) at 41-50 yrs. The comprehensive division of E.coli isolates is shown in Table 3.

Table 3: Age wise distribution of E.coli (n=215)

Age (Years)	No.of E.coli isolates	Percentage(%)
0-10	11	5.11
11-20	14	6.51
21-30	29	13.48
31-40	33	15.34
41-50	32	14.88
51-60	28	13.02
61-70	**53**	**24.65**
71-80	15	6.97
Total	215	100

Out of 215 isolates of *E.coli* 59 (27.44%) were found carbapenem resistant and 156 (72.55%) were carbapenem sensitive.

Table 4: Detection of Carbapenem Resistant E.coli.

Category	No. of E.coli (Percentage %)
Carbapenem Resistant E.coli	59 (27.44%)
Carbapenem Sensitive E.coli	156 (72.55%)

Discussion

The emergence of antibiotic resistance is aa issue of huge worry, predominantly in hospitals. It is observed that bacteria with antibiotic resistant seem to be biologically fit and able to cause severe life threatening infections. Enterobacteriaceae group is an example among gram negative bacteria which shows how bacteria can acquire, retain and put across new genetic information that can confer resistance one or more antibiotics. Hence resistance is a

severe problem among gram negative bacteria and hence needs for an efficient infection control measures to treat their spread.[17]

Currently, Carbapenem-resistant Escherichia coli is one of the main pathogen among Carbapenem resistant Enterobacteriaceae causing various clinical infections. [18]

In our study, distribution of E.coli isolates among different clinical samples revealed that, the frequency of E.coli was 20.47 % which was in the agreement wth the study of Atul garg et al (2018)]19], at SGPGI, Luck now where they also found prevalence of E.coli was 16%, while G ramesh kumar et al (2016)[20] reported 5% which is comparative low, while other studies reported high prevalence of E.coli in India such as Chirag et al (2021)[21] reported 31%, V. Niranjan et al (2014)[22] reported 56.8% in Puducherry, Mandira et al (2013)[23] were also reported 36% prevalence of E.coli. It shows that prevalence of E.coli vary from state to state as per geographical conditions.

Distribution of E.coli isolates among different clinical samples showed that the majority (53.95%) of isolates were from urine samples which was in cordance with the findings by Aggarwal et al(2012)[24] reported an isolation rate of E.coli from urine 50%, Shaswati et al (2014)[25] reported 65.32% and Wyawahare SA et al (2020)[26] reported 40.60%.

In our study it was observed that male (71.16%) was predominating among the patients in which E.coli isolates were obtained. It was may be because of the samples which were received from the male patients admitted in urology ward. Relatable type of studies were also observed in the past such as Nagoba and Wadher (1997)[27]. In their study they revealed that males (53.17%)were more as compared to female (46.82%). In one more study by Shobha et al. (2009) [28] also observerd the same pattern in which they observed that the males(54.37%) were more than female (45.62%). In another study of Ahmed et al. (2013) [29] it was revealed that the number of male patients (76.17%) than female (23.83%)

According to eh age wise distribution 61-70 years age group was dominating with 24.65% followed by the 31-40 years age with 15.34% and then 41-50years with 14.88% in our study which was having E.coli infection. Nagoba and Wadher[27] revealed that the maximum no. of E.colin isolates were found in 41-50 (79.31%) years age group and minimum in 0-10years of age group. In the the study of Mansour Amin et al (2009)[30] Mansour Amin et al (2009)[30] shown that the dominating age group was 21-40years age group, in Babu et al. (2014) [31] study the maximum no. of E.coli isolates were found in the same age group. While Ahmed et al. (2013)[29] revealed that maximum no. of isolates were found in 61-80 years of age group which was similar to our study.

In our study it was observed that the carbapenenm resistance E.coli isolates were obtained from 59(27.44%) from 215 E.coli isolates. Our study was in accordance with the study of R.A.Dahab et al (2017)[32] in which carbapenem resistant E.coli was observerd in 25.41% in Sudan, in the study of Hossein Kazemain et al, (2019)[33] the CRE were 27.7% in Iran and in a study in Hrayana by Namita Jaggi et al,(2019)[34] revealed 29.4% CRE. 26% CRE reported by Varaiya et al. (2008)[35], whereas 35% by Muley et al. (2013)[36] which was higher than our observation, 20.31% by Dwivedi et al. (2009)[37]and 13.42% by Bashir et al. (2011[38]which were lower as compare to our observation.

These results show that there is no consistency in the occurrence of carbapenemase producer at different places in the hospital in different studies. It varies always and this variation may be attributed to various factors, which govern the development of resistance in gram

negative bacteria commonly associated with various clinical infections.

Conclusion

Carbapenem resistant E.coli is fastly increasing and now this is the most important cause of trouble among the infectious diseases. Changing pattern of resistant need to be detected as early a possible to prevent the spread of resistant bacteria and to improve the strategies of treatment. Carbapenem resistant strains, pose serious problem choosing in the right antibiotic for treatment. In order to prevent carbapenem resistance to emerge in a hospital/health care setup, various strategies such as applying strict infection control measures, judicious prescribing of antibiotics, implementation of antibiotics resistance surveillance programs and antibiotic cycling must be done. Regular monitoring and documentation of carbapenem resistance should be done by all microbiology laboratories.

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Ethical Clearance

Approval from institutional Research Board and Institutional Ethical Committee (IEC Ref ID. MU/Research/EC/Ph.D/2018/24) was obtained before study commencement.

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