Carriage Of MDR *Escherichia Coli Isolated* From Stool Samples In A Tertiary Care Hospital

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ABSTRACT

The prevalence and antibiotic susceptibility patterns of intestinal carriage. 19 *E. Coli* isolates were labelled as multidrug resistant (MDR) because they were resistant to more than three classes of antibiotics. These 19 isolates were further tested for ESBL and MBL drug resistant detection. 3 were ESBL producers which was confirmed by Double disc synergy test and 7 were MBL producers confirmed by MHT. This study highlights the importance of performing the drug resistant detection against *E. coli* which helps in the proper management in MDR cases and also helps in infection of *E. Coli* among the patients admitted to tertiary care hospitals. A total of 50 stool samples were collected from the patients who are admitted within 3 days of hospitalization. The collected samples were processed for microscopy and culture for isolation identification & isolates were processed for Antibiotic susceptibility test and detection of drug-resistant (ESBL & MBL). Out of 50 *E. Coli* high resistance was found in 50 samples for ampicillin (68%), ceftriaxone (66%), ciprofloxacin (58%), and cefepime (54%). control program to reduce inappropriate antibiotic.

Keywords: Antibiotic Susceptibility test, Extended Spectrum Beta Lactamase (ESBL), Metallo beta lactamase (MBL), Double disc synergy test & Modified Hodge test.

Introduction

The species of the Escherichia genus is heterogeneous, and this genus includes both commensal and pathogenic bacteria. Although only some *E. coli* are pathological species, they cause infections in various organs, such as the urinary tract, biliary system, and central nervous system, ranging from spontaneously resolving cystitis to life-threatening sepsis syndrome in humans of all ages[1]. Increasing antibiotic resistance results in increased mortality and morbidity, enhances transmission of resistant bacteria, and increases health expenses[2]. Antimicrobial drug resistance is one of the most pressing global public health concerns of our time, threatening the effective prevention and treatment of infectious diseases in every country[3].

Several drug-resistant Gram-negative bacterial species belonging to Enterobacteriaceae have come to be designated by Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) to be "urgent-threat" or "priority 1" pathogens (Antibiotic resistance threat in United States ;2019). Major sources of drug-resistant Gram-negative bacteria include the environment such as contaminated water and food including meat and vegetables and healthcare settings[4]. Additionally, intestinal commensal drug-resistant bacteria have been reported as an important reservoir of antimicrobial drug resistance genes (ARGs). E. coli is a member of the commensal flora of human and other warm-blooded animal intestinal tracts. As such, they can acquire ARGs by horizontal gene transfer from drug-resistant E. coli strains and other Gram-negative bacteria that enter the intestinal tract via exposures to contaminated food, water, and other external sources[5]. E. coli can be transmitted through contaminated water or food, or through contact with people and other animals. Thus, risk factors for fecal carriage of drug-resistant commensal E. coli and ARGs could include exposures to environmental sources of drug-resistant bacteria, in addition to traditional risks such as prior use of antibiotics or healthcare-associated infections[6].

The rapid rise of multidrug-resistant (MDR) bacterial infections is a major public health concern and a growing threat to the global health security. Unregulated use of broad-spectrum antibiotics and widespread reservoirs of these pathogens are main contributors to this problem[7]. Broad spectrum antibiotics, in particular third generation cephalosporin's (3GC), are among the most frequently prescribed drugs for the treatment of infections caused by Enterobacteriaceae[8]. Failure of treatment with these antibiotics has increasingly been reported due to the emergence of extended spectrum beta-lactamases (ESBLs) during the last two decades[9]. Several studies have shown that patients are more likely to be exposed to antibiotics directly through the administration of antibiotics to avoid other infections. This consumption of antibiotics affects the intestinal microflora of the patients[10].

Resistance to antimicrobial agents has become a clear and present global threat. Different antibacterial agents are rendered ineffective due to the growing number of resistant bacteria, which are constantly acquiring different resistance mechanisms. Extended-spectrum beta-lactamases (ESBL), are considered as a cornerstone in the antimicrobial resistance problem[11]**.** Beta-lactam resistance in Gram-negative bacteria is mainly mediated by ESBL enzymes. They are encoded by genes that are often located on transferable conjugative plasmids. Additionally, genes that confer resistance to antibacterial agents, other than beta-lactams, are frequently carried simultaneously on these plasmids and contribute to the dissemination of resistance[12]. In early 1990s, ESBL-producing Gram-negative bacteria used to be confined to hospitals and intensive care units (ICU), however, this soon changed with the occurrence of infections caused by ESBL-producing E. coli in the community.

The increasing and rapid spread of Metallo-beta-lactamase (MBL) producing Enterobacteriaceae, particularly Escherichia coli and represents an emerging public health threat. Over the past few years, Metallo-beta-lactamase (MBL) producing gram negative bacteria are being reported with increasing frequency from several parts of the world and have emerged as a most widespread and clinically significant carbapenem resistance mechanisms. We have undertaken this study to ascertain the incidence of MBL production in clinical isolates of E coli at a tertiary care hospital in Mysore.

Materials & Methods

This study was conducted in the department of Microbiology, JSS Hospital in a period of 3 months. A total of 50 stool samples were collected from 50 patients located in the selected

wards using sterile stool containers provided to all patients in the date of interview. Exclusion criteria were any history of antibiotic treatment or hospitalization. Clinical details and demographic characteristics (age, gender and place) of the patients were collected along with the informed consent. The fecal samples were processed within 1to 2 hours of collection. The collected samples were subjected for direct microscopy and culture. Samples were inoculated onto MacConkey agar and HEA medium for isolation and the isolates were further identified by using standard biochemical reactions. All the isolated and identified Escherichia coli were further subjected to antibiotic sensitivity testing by Kirby-Bauer disc diffusion method as per CLSI guidelines. Tested antibiotics were Ampicillin, Ceftriaxone, Cefepime, Amoxiclav, Ertapenem, Amikacin, Tetracycline, Gentamycin & Ciprofloxacin. The isolates were further processed for phenotypic detection of ESBL and MBL production.

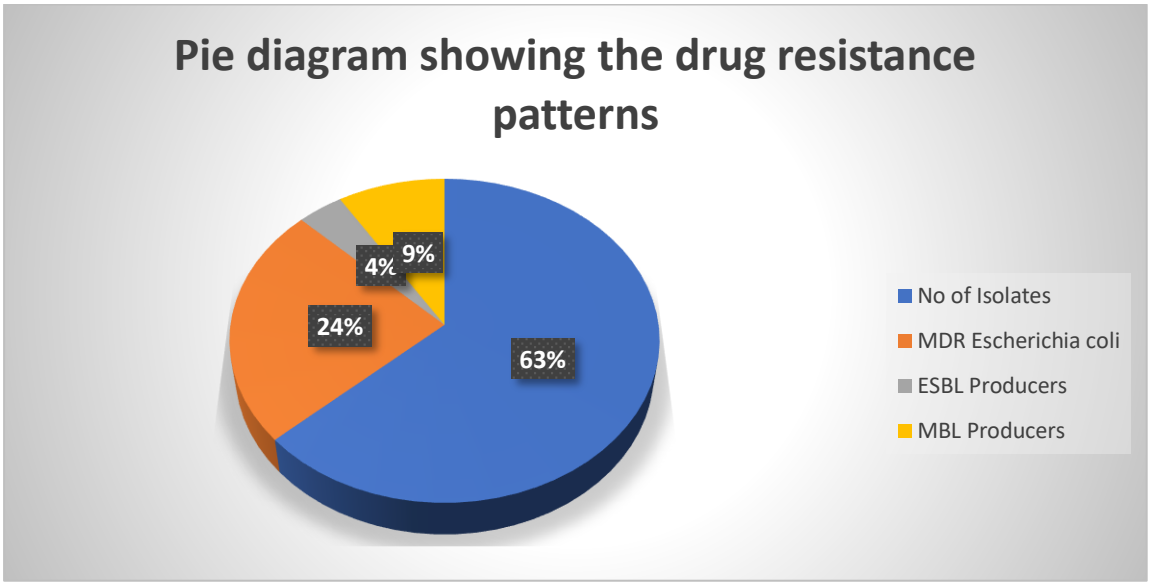
Results

A total of 50 stool samples were collected from patients, JSS Hospital. Out of 50 samples 28(56%) were males and 22(44%) were female. In 50 stool samples 13 were collected from the age group 31-40, again 13 were from 51-60, 6 from 61-70, 5 were from 10-20, 5 were from 21 – 30, 3 were from below 1, 3 were from 71-80, 2 were from 41-50. Among 50 isolates 90% & 96% were sensitive to Amikacin and Gentamycin followed by Ertapenem and Amoxiclav. Among 50 isolates 68% were Resistant to Ampicillin followed by Ceftriaxone and Ciprofloxacin **(Table 1)**. Out of 50 *Escherichia coli* isolates 38% (19 /50) isolates were MDR, out of 50 *E. coli* isolates only 6% (3 /50) were confirmed as ESBL producers, where as 14% (7/50) were confirmed as MBL producers **(Chart 1)**.

Table 1 showing Antibiotic sensitivity patterns of 50 isolates

Antibiotics	**Sensitive %**	**Resistant %**
Ampicillin	16 (32%)	34 (68%)
Ceftriaxone	17 (34%)	33 (66%)
Cefipime	23 (46%)	27 (54%)
Amoxiclav	31 (62%)	19 (38%)
Ertapenem	34 (68%)	16 (32%)
Amikacin	46 (90%)	05 (10%)
Tetracycline	26 (52%)	24 (48%)
Gentamycin	48 (96%)	02 (4%)
Ciprofloxacin	21 (42%)	29 (58%)

Pie chart 1 showing the drug resistance patterns of 50 isolates



Discussion

This study identified the fecal carriage of Multidrug Resistant Escherichia coli from stool sample among the patients of tertiary care hospital JSS hospital, Mysore. As far as we know, this is the study to identified the carriage of multidrug resistant Escherichia coli in the patients admitted to the hospital. Previously, our fecal sample collection and surveillance conducted at the JSS hospital from April to May 2022 identified the prevalence of drug resistant E. Coli among the volunteers from fecal. (Rubin J, Mussio K, Xu Y Suh J, Riley LW; 2020). In the present study, we aimed to identified multidrug resistant Escherichia coli from the patients who admitted before 2 days of sample collection.

Overall, the prevalence of drug resistant E. Coli was slightly low in our population, with 38% (19/50). The rates of resistance to first-line antimicrobial agents, namely Ampicillin & Amoxiclav and first-generation cephalosporins, such as Ceftriaxone and Cefepime were–68%,38%,66% & 54% respectively. Furthermore, the rate of resistance to ciprofloxacin was 58% of E. Coli were ESBL producers. The aforementioned studies had varied methodologies, study periods, sample sizes, and demographics and should thus be compared cautiously. In the present study, resistance to the extended spectrum cephalosporins ranged from 66% to 54%, while resistance to Ertapenem was 32%. Amikacin and gentamicin show sensitivity with the 10 %and 4% respectively.

MDR bacteria are defined as bacteria resistant to at least one antimicrobial agent from at least three different antimicrobial categories (Magiorakos AP, Srinivasan A, Carey RB, et al;2012). In this study 19 out of 50 E. Coli isolates were MDR. (Baljin et al) reported that the fecal carriage of MDR Gram-negative bacteria, in their study, ranged from 42.2% to 69.2%, while in another study, (Huang et al.) found that MDR E. Coli strains accounted for 37% of the isolates in the study. A higher resistance rate was reported in another study in Bangladesh, the presence of MDR E. Coli among fecal E. Coli resistant to the third-generation cephalosporin was 77% (Islam MA, Amin MB, Roy S, et al; 2019). Using phenotypic method, 3 out of 50 isolates were Detected to be ESBL producers. In present study there were only 15.7% ESBL producers are identified from 19 MDR isolates. Similarly, in a study in France, Janvier et al. Reported that all their Gram-negative ESBL isolates were MDR (Janvier F, Merens A, Delaune

D, et al;2011). However, in an earlier study conducted in Egypt, Abdallah et al. Reported that only 47.83% of the ESBL positive Gram-negative isolates were MDR (Abdallah HM, Alnaiemi N, Reuland EA, et al;2017).30 out of our 70 (42.86%) E. Coli isolates were ESBL producers. Fourteen out of the 35 (40%) E. Coli isolates collected at time of hospital admission were ESBL producers. After hospital admission, ten of these E. Coli isolates remained ESBL producers, while another six isolates became ESBL producers, so that the total number of ESBL-producing isolates in group B (after hospital admission) became 16 out of the 35 (45.7%) (Amira ElBaradei, Dalia Ali Maharem and Iman S. Naga; 2020).

However, in case of metallo-beta-lactamase producing Escherichia coli using phenotypic method, 7 out of 50 isolates were identified as MBL positive. In present study there were 36.84% MBL producers are identified from 19 MDR isolates. In the early study, AMR, multi-drug resistance (MDR) and ESBL production were observed in more than 54.9, 36.2 and 11.7% of commensal E. Coli isolates, respectively. Out of six isolates resistant to imipenem and meropenem, four isolates were phenotypically detected as MBL producers. (Fahimeh Mahmoodi1, Seyedeh Elham Rezatofighi1* and Mohammad Reza Akhoond2; 2020). In another study Enterobacteriaceae into the paediatric community has followed. Reports suggest that this occurred as early as 2002. Here, we reflect on the unwelcome emergence of MBL-producing Enterobacteriaceae in US children and the available clinical and molecular data associated with spread. Since 2002, there have been disturbing reports that include the most readily transmissible MBLs, blaIMP, blaVIM, and blaNDM types. In the majority of children with available data, a history of foreign travel is absent. (Latanina K logon, Robert A Bonomo; 2016). The frequency of CPE fecal colonization in our study is higher than the clinical prevalence previously found in Spanish hospitals (Miró E, et al. 2010.), although it is still lower than that reported in another study in hospitalized patients in France during a nonoutbreak period (5.3%) (Vidal-Navarro L, Pfeiffer C, Bouziges N, Sotto A, Lavigne JP. 2010.). Colonization of patients from the community setting with CPE, all of them MBL producers, is also remarkable. These results are an alert for a hidden fecal carriage of MBL-producing isolates, as a high percentage of the isolates from positive fecal carriers (82%, 9 of 11 patients) were not infected with CPE isolates. (Desirèe Gijón, Tânia Curiao, and Rafael Cantón;2012).

Conclusion

Multidrug resistant *E. coli* is a known threat in treating patients, we have arrived at dead end where there are very few antibiotics left in the pipeline for the treatment of MDR organism. In this regard testing of isolates from patients should compulsorily involve ESBL, MBL & other resistance mechanism detection also along with regular antibiotic susceptibility testing.

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