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Emergence of carbapenems resistance in *Escherichia coli* isolated from different clinical samples at University Hospital of Pakistan

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Abstract

Escherichia coli are Gram negative, facultative anaerobic bacteria. It constitute about 0.1% of normal gut flora of Human and is commonly found in lower intestine. In this study we have determined the frequency of carbapenems resistance in *Escherichia coli* in different clinical isolates by simply disk diffusion method. This study was conducted from 26th January 2010 to 29th December 2011, in Ziauddin university hospital Karachi Pakistan. Sum of 2710 consecutive clinical isolates of *Escherichia coli* were collected and identified by conventional microbiological techniques. Antimicrobial susceptibility testing and all ESBL positive bacteria antibiotic susceptibility pattern to meropenem (10 ug), and imipenem (Becton Dickinson, USA) (10 ug) was recorded for this study. By Kirby-Bauer method, as per NCCLS guidelines, isolates were considered as resistant to carbapenems. A total 2710 isolates of *Escherichia coli* were obtained during the study period. The isolates from females patients were 1716/2710 (63.32%), while the isolates from male patients were 994/2710 (36.68%). Female to Male ratio was 1.73:1. In these total isolates the number of Carbapenems resistant *Escherichia coli* is 13 which is 0.48%. The emergence of metallo-beta lactamases producing *Escherichia coli* which are resistant to carbapenems are becoming a severe therapeutic problem worldwide. There is strongly need to emphasize on the rational and judicious use of antimicrobials and adhere to the concept of "reserve drugs" to minimize the misuse of available antimicrobials. In addition, more effective infection control measures and regular antimicrobial susceptibility is also an essential component in reducing the emergence of these resistant organisms and should be prompted.

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Introduction

Escherichia coli is a Gram negative, facultative anaerobic bacterium. It is non-sporulate, typically rod-shaped bacteria and its size is about 2.0 micrometer long and 0.5 micrometer in diameter, with a cell volume of 0.6-0.7 micrometer (Kubitschek, 1990). *Escherichia coli* constitute about 0.1% normal gut flora of human and is commonly found in lower intestine. The major route of transmission of pathogenic *Escherichia coli* is the fecal-oral transmission (Eckburg *et al.*, 2005). The Pathogenic strain of *Escherichia coli* is responsible for variety of infection. It may cause about 90% of urinary tract infection in individual without any structural defect in urinary tract (Todar, 2007). It also causes bacteremia, septicemia, biliary infections, liver abscess, upper respiratory infections, wound infections, otitis media, neonatal sepsis, and neonatal meningitis.

Antibiotics resistance is a world side growing problem, some of these are due to non judicious use of antibiotics by human, and use of antibiotics in growth promoters of animal feed has also contributed in development of resistant pattern in microorganisms (Johnson *et al.*, 2006). According to a study in 2007 the adaptive mutations in *Escherichia coli* in order of 10-15 per genome per generation, this finding is 1000 times high than previous estimates this finding have a significance role for the management of antibiotic resistant in bacteria (Perfeito *et al.*, 2007). The bacteria which have acquired resistance to Beta-lactam antibiotics become more common in the recent decades, and it's a particular problem for the community (Paterson *et al.*, 2005). The Beta-lactamases enzyme which produces by *Escherichia coli* makes much penicillin and cephalosporin not all, infective during antibiotic therapy. *Escherichia coli* which produce extended spectrum Beta-lactamase is highly resistant to much class of antibiotics, which strains are very difficult to treat. One of two oral antibiotics and a very limited group of intravenous antibiotics are remain effective for the treatment of resistant strain of *Escherichia coli*.

In December 2009 a gene detected in Gram-negative bacteria which name is New Delhi Metalo Beta-lactamases (NDM-1), this gene is detected in a patient sample of Swedish national that he acquired in India during traveling, the NDM-1 strains give even resistant to carbapenems class of antibiotics, which is the drug of choice for previous resistant strains (Yong *et al.*, 2009).

NDM-1 metallo beta lactamase is an a type of enzyme that makes *Escherichia coli* resistant to wide range of antibiotics having Beta-lactam ring, in which carbapenems are also included. The resistance to carbapenem is because of gene named as bla NDM-1 (Yong *et al.*, 2009). *Escherichia coli* having NDM-1 carbapenemases are highly resistant to many classes of antibiotics, which include Fluroquinilones and Aminoglycosides; the main classes of antibiotics for the treatment of Gram-negative infections, a few isolates remain sensitive to Polymyxine and Tegacyclin. In this context the detection of such isolates of *E.coli* has been done in order to have a better understanding regarding the prevalence of such strains in clinical isolates of a tertiary care hospital which can helps in therapeutic management, as well as impherically treatment of such resistant strains. This study highlights the increasing resistance in *E.coli* towards Meropenem and Imepenem in our country.

Materials and methods

Samples Collection

This study was conducted from 26th January 2010 to 29th December 2011, in department of Clinical Microbiology, Ziauddin university hospital Karachi Pakistan. Total of 2710 consecutive isolates of *Escherichia coli* were collected from clinical samples, by convenient sampling. Sources were Urine, blood, respiratory secretions, wound swabs, Pus in Syringe, Pus Swabs, Cerebrospinal Fluid, Ascitic Fluid, Peritoneal Fluid, Bronchial Alveolar Lavage, Sputum, Tissues, Fine Needle Aspiration, Pericardial Fluid, Bone, Semen, Ear Swab, Liver Abscess, Bile, Eyes Swab, central venous pressure (CVP) lines tips, and Different other sources. Approval from the hospital

ethical committee was obtained. Information was taken from either the patient or from any other patient's relative.

Sample processing

All clinical samples were received in a sterilize container or in an Amies transport medium supplied from the microbiology laboratory. All These clinical samples were inoculated on Routine Medias, Sheep Blood agar, Chocolate agar and Mac Conkey's agar, Sheep Blood agar and Chocolate agar was incubated at 37°C in CO₂ incubator and Mac Conkey's agar in ambient air for 24-48 hours, by applying standard microbiological techniques. *Escherichia coli* were identified using conventional techniques (colony morphology, gram staining, Indole test and Fermentation of sugars on TSI Medium) (Koneman *et al.*, 2006).

Antimicrobial susceptibility

Antimicrobial susceptibility testing was performed on Mueller Hinton agar (MHA) (oxide Ltd., England) using modified Kirby Bauer's disk diffusion method according to clinical and laboratory standards institute (CLSI) guidelines. A suspension was made of organism equal to 0.5 McFarland and inoculated onto a MHA plate. The antimicrobial discs used were, Amikacin 30 ug, Amoxycillin/Clavulanic acid 30ug, Ampicillin 10ug, Cefixime 5ug, Trimethoprim 25ug, Cefotaxime 30ug, Nitrofurantoin 300ug, Gentamicin 10ug, Ofloxacin 5ug, Imipenem 10ug, Meropenem 30ug, Cefipime 30ug, Tazobactam 110ug, Cefpirome 30ug, Carbimicilin 100ug, Ceftazidime 30ug. Referring to the recommendations of NCCLS guidelines interpreted results (Cheesbrough *et al.*, 2000). Local antibiotics Polymyxin B 300ug, Tigecycline 15ug, Colistin sulphate 10 ug against gram negative bacilli. American Type of Culture collection (ATCC) Controls will be used to check the quality of media and antibiotic disc. *E.coli* ATCC 25922 (Beta-lactamase negative) strains were used as a control organism.

Carbapenem Resistant Detection

All Gram-negative bacteria which were isolated from

these clinical samples were tested for ESBL production by using four disks (concentration in µg) ceftazidime (30), ceftazidime/clavulanic acid (30/10), cefotaxime (30), and cefotaxime/clavulanic acid (30/10) and interpreted as per NCCLS guidelines. In all ESBL positive bacteria antibiotic susceptibility pattern to meropenem (10), and imipenem (Becton Dickinson, USA) was recorded for this study. By Kirby-Bauer method, as per NCCLS guidelines, isolates were considered as resistant to carbapenems such as meropenem and imipenem if the zone of inhibition was < 13 mm. The intermediate 14-15 mm and the sensitive for these antibiotics are > 16 mm.(fig 1)

Statistical analysis

Data was analyzed by using Statistical Package for Social Sciences (SPSS) software version-16.0. The frequencies of Carbapenem Resistant *Escherichia coli* were calculated and expressed in percentages.

Results

A total 2710 isolates of *Escherichia coli* were obtained during the study period. Distribution of Isolates from different simple source is shown in fig 2. In total isolates the numbers of urinary isolates were 64% (n=1728), Blood 8% (n=204), Pus 17% (n=466), Pleural fluid 11% (n=12), Respiratory samples 6% (n=153), CSF 0.11% (n=03), Liver abscess 0.33% (n=09), Ascitic fluid 1% (n=21), Tissue 0.25% (n=7), Bile 0.29% (n=8), Peritoneal fluid 1.2% (n=35), Wound swab 2% (n=64). Predominantly the isolates were from females patients 1716/2710 (63.32%), while the isolates from male patients were 994/2710 (36.68%). Female to Male ratio was 1.73:1. In these total isolates the number of Carbapenems resistant *Escherichia coli* was 13 which is 0.48%. In total 13 carbapenems resistant the male patient were 0.6% (n=6) and female patient were 0.4% (n=7). Carbapenems resistant rate in male and female patient's shows in Table 1.

Discussion

Carbapenems such as imipenem and Meropenem are class of beta-lactam antibiotics, which act by

inhibiting cell wall synthesis, have a broad spectrum of antibacterial activity, and therefore is used empirically as well as direct treatment according to result and sensitivity testing, in treatment of severe nosocomial infection in critically ill patients of ICU or after therapeutic failure of first line antibiotic for

gram negative bacteria. The special chemical structure of carbapenems makes them highly resistant to most beta-lactamases (Philippe *et al.*, 1999). *E.coli* produced various beta-lactamases which renders them resistant to various beta-lactam antibiotics eg; penicillins and cephalosporins.

Table 1. Carbapenem resistant rate in male and female.

S.N	Gender	Total Isolates	Carbapenem Resistant	Percentage
1	Male	994(36.6%)	06(46.1%)	0.6%
2	Female	1716(63.3%)	07(53.8%)	0.4%
	Total	2710	13	0.47%

Carbapenems are generally resistant to typical beta-lactamases, but rarely there has been an alarming rise in spread of carbapenems resistance in *E.coli*, due to production of metallo beta-lactamases. The emergence of carbapenems resistant enteric bacteria eg; *E.coli* have been isolated from Pakistan, Bangladesh and India (Baquero *et al.*, 1997). Carbapenems which are considered to be the drug of choice for treating for serious nosocomial infections caused by most Gram-negative organisms. The emergence of resistance against these classes of antibiotics is a cause of concern. There are several reasons which have played the part in this situation which include non-judicious use of carbapenems; inappropriate duration of antibiotics and sub therapeutic concentrations of the drug is also another important cause for emergence of resistance (Patel *et al.*, 2008; Livermore and Woodford., 2000). Gram negative pathogens which are acquired carbapenems resistant are difficult to treat, and have a significant impact on patient's morbidity and mortality. Poor infection control practices also can be blamed for acquired carbapenems resistance (Roberts. 2010). Inefficient contact precautions and cohorting of patients as well as unawareness of proper infection control practices have also contributed to this scenario. Unidentified colonize patients have serve as a potential reservoir for transmitting carbapenems resistant strains (Calfee and Jenkins, 2008).

In our study a total of 2710 *Escherichia coli* were isolated from different clinical samples the majority

of isolates were from Urine culture sample that are 64%. A study from Kanpur India Shivesh Prakash also reported high percentage of urinary isolates in his study which is 54% of total isolates (Shivesh. 2006). The majority samples of our study are from critical units such as Intensive care unit (ICU), Cardiac care unit (CCU) and Neonatal intensive care unit (NICU), the most prevalent were from ICU patients that are 492/2710(18%). A study from India in 2006 reported the most prevalent isolates from ICU patients which are 469/2626 (17%)(Gupta *et al.* 2006).

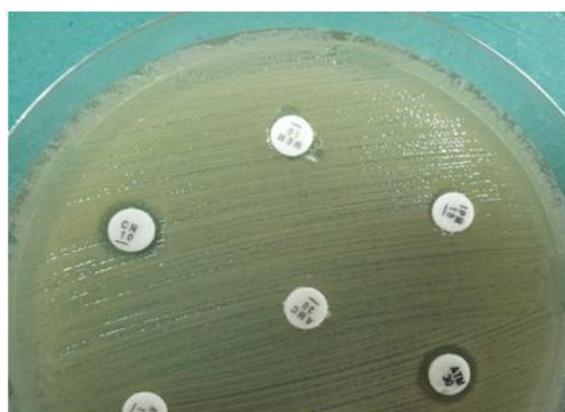


Fig. 1. Showing Zone of Inhibition of Imepenem (IPM), and Meropenem (MEM) for the detection of Carbapenem Resistant *E.coli*.

We found in our study that incidence of resistance against Meropenem was equal to Imipenem. The frequency of carbapenems resistant *Escherichia coli* is 0.48% in our study. A study from India reported 2.1% (Gupta *et al.* 2006), and from Taiwan reported 0.09% carbapenems resistant in *Escherichia coli* (Yi-Fang *et al.*, 2008).

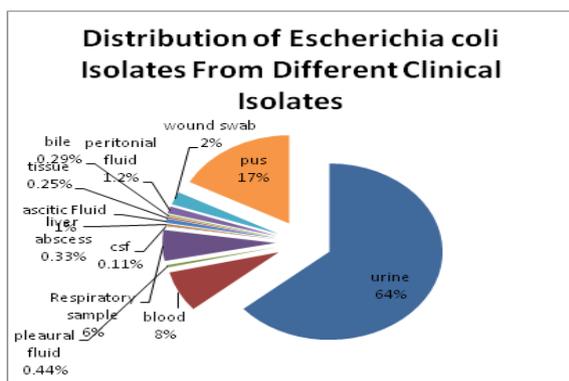


Fig. 2. Distribution of Isolates of *Escherichia coli* from different clinical samples.

The major cause of isolation of carbapenems resistant strains in hospital is due to irrational use of antimicrobial and improper infection control practices. Though frequency of carbapenems resistance in *E. coli* is less at this moment but due to non judicious use of antibiotic, because use of extended spectrum cephalosporins can lead to a selective pressure in emergence of carbapenems resistant *E.coli*. Using of carbapenems such as Meropenem and Imipenem without culture and sensitivity will lead in selecting out of carbapenems resistant strain of *E. coli*, which would be a serious problem as the therapeutic options to treat such infection will be limited. Based on currently available information, infection control measures should be taken on emergency basis to stop the spread of carbapenems resistance in *E.coli*, both at local and international levels. Health care facilities should have written plain as well as should strongly stick to infection control guideline in order to limit the transmission of carbapenems resistant *E.coli*. Special attention should be given on detection as well surveillance of this emerging resistance pattern, because a serious medical problem may occur, due to therapeutic failure if such strains become common in health care facilities.

Conclusion

The emergence of metallo-beta lactamases producing *Escherichia coli* which are resistant to carbapenems are becoming a severe therapeutic problem worldwide. Mostly these isolates have a common multidrug-resistant pattern that not only includes

carbapenems, but also the other classes of essential antibacterial agents, Cephalosporins, Aminoglycosides and Fluoroquinolones. There is strongly need to emphasize on the rational and judicious use of antimicrobials and adhere to the concept of "reserve drugs" to minimize the misuse of available antimicrobials. In addition, more effective infection control measures and regular antimicrobial susceptibility is also an essential component in reducing the emergence of these resistant organisms and should be prompted.

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