Genotypic and Phenotypic Characterization of Carbapenam Resistance Enterobacteriaceae through Amplification of Carbapenam Resistance Genes

Azka Rizvi[1*], Dr. Sana Fatima Dogar[2], Sidra Noureen[3], Isra Khalid Rnan[4], Ramsha Shoaib[5], Dr. Fauzia Qureshi[6]

1. Institute of Microbiology, University of Veterinary and Animal Science, Lahore.
2. Department of Pathology, Shalamar Medical and Dental College, Lahore.
3. Department of Microbiology and Molecular Genetics, the Women University Multan.
4. Medical Officer
5. Department of Molecular Biology, Virtual University of Pakistan.
6. Associate Professor of Anatomy, Akhter Saeed Medical and Dental College, Lahore.

Corresponding Author:
Azka Rizivi
Email: azka.rizvi528@gmail.com

Abstract

Over the past few years, antibiotic resistance has become a global issue. Use of broad spectrum antibiotics and antibiotic abuse has made antibiotic resistance a major health concern and there is need to address it. Enterobacteriaceae is the most infectious group of pathogens which are usually handled with broad-spectrum antibiotics like carbapenam and beta lactams. Resistance to these groups has reached an alarming level. This Cross-sectional study focused on carbapenam resistant Enterobacteriaceae (CRE) and ESBL producing bugs. For this, samples from 492 clinically suspected cases of bacterial infections were collected including blood, sputum, urine and Pus samples. After isolation of bacterial growth, identification and sensitivity was tested using Vitek 2 compact. For carbapenam resistance testing, samples were subjected to genotyping by GeneXpert system - CarbaR method. Highest infection rate was recorded among male patients and *E.coli* was the most common pathogen. Although rate of ESBL was similar among all the participating departments, highest CRE rate was recorded from pediatric ward's patients (94%) followed by orthopedic (89%), general OPD (89%) and ICU (67-70%). National institute and other policy makers can utilize these findings in channelizing and regulating the administration of antibiotics to the infected individuals in order to combat increasing antimicrobial resistance in the country.

Key words: Carbapenem Resistance, Extended-spectrum beta-lactamase, antibiotic resistance, carbapenemases, beta-lactamases.

Tob Regul Sci. ™ 2022;8(1): 3687-3695
DOI: doi.org/10.18001/TRS.8.1.279

INTRODUCTION

Antibiotics resistance is an emerging and alarming situation all around the World. Healthcare set-up are very much involved in spreading antibiotics resistance by either misuse of antibiotics or wrong prescription, more importantly the lack of education, knowledge and awareness about use of antibiotics is considered a key factor for spreading of drug resistance among the bacterial pathogens(1). Countries with low literacy and less sources are more affected with antibiotic resistance (2). Among the antibiotic resistance bacteria gram negative bacteria are more prevalent than gram negative (3). And in these gram negative Enterobacteriaceae is always a more concern because in this class of bacteria a major group is normally present in human gut flora. Resistance of cephalosporin, aminoglycosides, fluoroquinolones and carbapenam is increasing with time very rapidly (4). They are the source of a variety of illnesses, such as infections of the bloodstream, urinary tract infection, wound infection and lower respiratory tract infection. Antibiotics belonging to the beta-lactam class are typically used to treat infections of this kind. Examples of antibiotics belonging to this class include Cephalosporin, Penicillin, Carbapenam and Monobactams (5). Carbapenam-resistant Enterobacteriaceae (also known as CRE) are bacterial strains that have developed resistance to the antibiotic class known as carbapenam, which is commonly used to treat severe infections. CRE are resistant to the vast majority of the other antibiotics that are typically used, and in some circumstances, they are resistant to all of the antibiotics that are available (6). Carbapenam have been an essential antibiotic class for the treatment of these pathogens, and resistance to carbapenam among Enterobacteriaceae in the developed countries has been uncommon up until quite recently. On the other hand, the appearance of novel beta-lactamases that have direct carbapenam-hydrolyzing activity has led to the rise in the prevalence of carbapenam resistant Enterobacteriaceae (CRE). Given the prevalence of illnesses that are caused by Enterobacteriaceae, CRE pose a particularly difficult challenge and it is associated with increased hospital stay, increase cost of treatment and high mortality specially in health care set-up (7). In 2013 according to disease control program only in USA there were 35000 morbidities and 2000 mortalities registered (8). These days CREs are progressively being identified all over the world, and as a result, they are developing into pressing clinical concerns. Consequently, the future danger lies in the potential for these organisms to develop pan-drug resistance as they progress from multiple drug resistances. Therefore, information on -lactamase-producing Enterobacteriaceae is absolutely necessary, and this is the one and only means to keep the antibiotics of last resort effective (9). And purpose of this study is to find the prevalence of carbapenam resistance in Enterobacteriaceae also to check either the resistance is induced or genetically acquired. this will aware the seriousness of this situation and make policy makers to bound for adaptation of some strategy to overcome and stop spreading these carbapenam resistance bacteria.

Material Method

Study Design

This was a cross-sectional study conducted December 2021 to April 2022 at a tertiary care hospital in Lahore, Pakistan. Participants of the study were selected on the basis of clinical suspicion of bacterial infection. To avoid duplication of the samples, one type of sample from each patient was collected.

Ethical considerations

Ethical aspects were also considered and for that institutional ethical review board's approval was also obtained. Patient/participant's credentials were kept confidential and no personal information was revealed during or after the experiments. Potential benefits and scope of study was explained to the study participants, and in case of minor (age <18), to their legal guardians. Duly filled consent forms were obtained from study participants.

Data Collection

After explaining benefits and harm of the study to the participants and obtaining their consent, participants were also asked to fill out pre designed questionnaires to get their demographic characteristics and other details (as per study design). In case of unconscious patients, their attendant or guardians were asked to fill out forms.

Sample Collection

Total 492 patients from different departments of the hospital such as surgery, nephrology, urology, and gastroenterology were identified with symptoms of bacterial infection. Blood, Sputum, Pus, wound swab and urine samples were collected from these patients. One type of sample from one patient was collected in a sterile container. Samples were immediately transferred to the laboratory for further processing as per protocol.

Sample Processing

All samples were inoculated on three agar media's. Blood agar for growth promoting chocolate agar for fastidious bacteria and MacConkey agar for selective gram negative bacteria also to differentiate the lactose fermenter and non-lactose fermenter.

Urine Sample was inoculated on CLED agar with 10ul loop to consider more than 100 colony forming units to be significant growth.

Blood culture was collected in ***BactAlert***® media plus culture bottles in aerobic and anaerobic conditions which will be later on subculture on given three agar media's.

Sputum sample must sputum no saliva contamination should be present

Wound sample must be preferred as wound discharge, swab was least priority

Growth Identification

After 24-48 hours of incubation, positive samples were processed for identification. Organisms were identified using bench tests such as catalase, coagulase and oxidase. All isolated organisms were further identified using Vitek-2 Compact which is based on phenotypic character identification having 100% sensitivity and 99% specificity (10). Identification cards were used having reference number gram negative ID21341 and gram positive ID21342.

For this, McFarland was prepared using DensiCheck of Vitek-2 instrument and 0.5-0.63 was considered as acceptable range for standard McFarland. After preparation of the suspension, ID card was inserted in the suspension followed by loading on to the system. Each sample was given a unique identifier and results were recorded after 24 hours. To check purity of the tested suspension, this suspension was inoculated on to the blood agar followed by incubation period of 24 hours. Test was repeated if the purity was not up to the mark i.e. only single type of colonies were not isolated.

Antimicrobial Sensitivity Testing

Antimicrobial sensitivity testing was done as per CLSI guidelines 2022. AST-N-222 sensitivity cards were used to check sensitivity of all gram negative bacteria. Total 5 classes of antibiotics were tested including Cephalosporin, Carbapenam, Aminoglycosides, Fluoroquinolones and colistin. Sensitivity was measured as minimum inhibitory concentration.

For this, 0.5 McFarland of the organism to be tested was prepared on DensiCheck instrument. From this sample 145µl (in case of gram negative isolates) and 245µL (in case of gram positive isolates) transferred into another tube containing only the Vitek saline. After that, respective cards were inserted into the tubes and these prepared samples were loaded on to the machine. After 24 hours results were noted.

Confirmation of Genes by GeneXpert

Simple resistance was measured with antibiotics and genetic resistance detected with PCR testing, Cartridge base PCR by GeneXpert system was used with Carb-R cartridge having 99% sensitivity and 100% specificity (11).

Quality control testing

To check antimicrobial sensitivity, the ATCC quality control were used with all drugs and used for pathogens and same identification technique used for pathogen isolated. Reference strains of *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853) *E. faecalis* (ATCC 29212).

Data analysis

Analysis of the Data Daily entries of the data that were created were made into Epi-data version 3.1, which was followed by import into Statistical Package for Social Science (SPSS) version 22.0 and analysis using that program (IBM USA). Calculated and summarized descriptive data reveal the frequency of demographic parameters, the size of Enterobacteriaceae infections, and the medication resistance profile. These statistics were presented in the form of graphs and tables.

Results

Demographic characteristics

A total of 492 patients were recruited in the study based on the demographic distribution as given in table 1. Highest number of patients belong to the age group 20-50, which is 139 males and 111 females. Lowest number of patients were recorded in younger age group i.e. <10 years which is 11 patients in total.

Table 1 Demographic distribution

Age group	Male	Female	Other	Total
< 10 years	7	4	0	11
10-20 years	41	37	1	79
20-50 years	139	111	3	253
> 50 years	60	89	0	149

Sample Distribution

From 1157 total samples collected from 492 patients, maximum number of samples were recorded for blood cultures. Positive cultures of different samples distribution were given as

Table 2 Positive samples distribution

Patient location	Blood culture	Urine	Pus/Wound	Sputum	Fluid
OPD	16	12	3	1	1
ICU	30	16	7	3	3
Orthopedic	15	12	2	2	4
Surgery	31	6	4	2	3
Pediatrics	23	8	2	1	0
Total	115	54	18	9	11

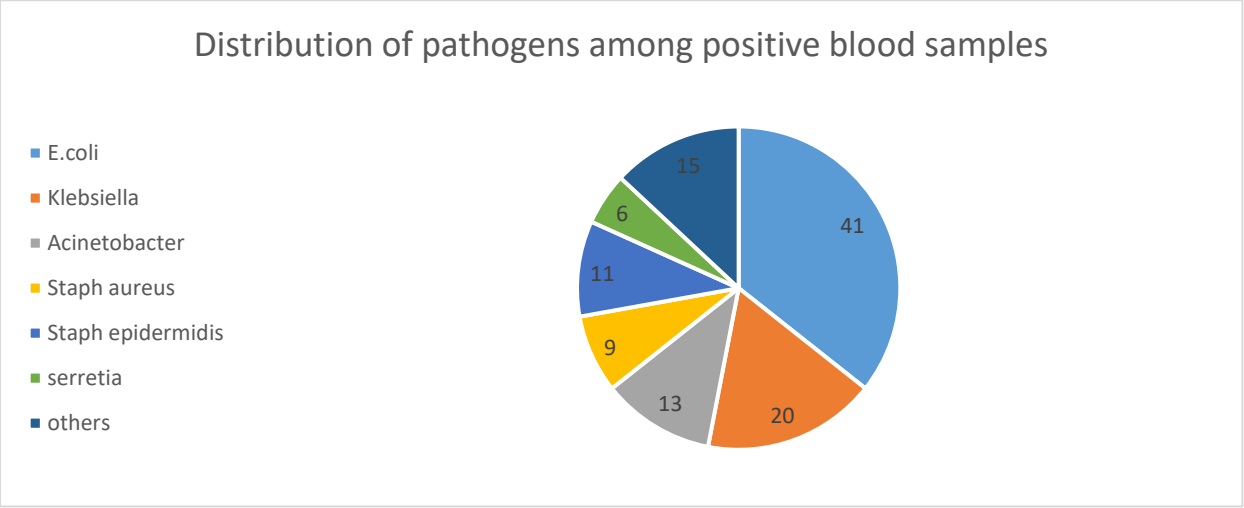

Figure 1 pathogen distribution

In total 1157 samples 207(17.9%) were positive out of which most isolated organism was *E.coli* which were 83 (40%) from total isolates after that *Klebsiella pneumoniae* was 41 (19.8%) and third highest was *Staph aureus* 28 (13.5%). Other pathogens in total were 55 including *Acinetobacter* Spp, *Serretia marscence*, *Burkholderia cepacia*,*Citrobacter* Spp, *Klebsiella oxytoca* and *staphylococcus epidermidis*.

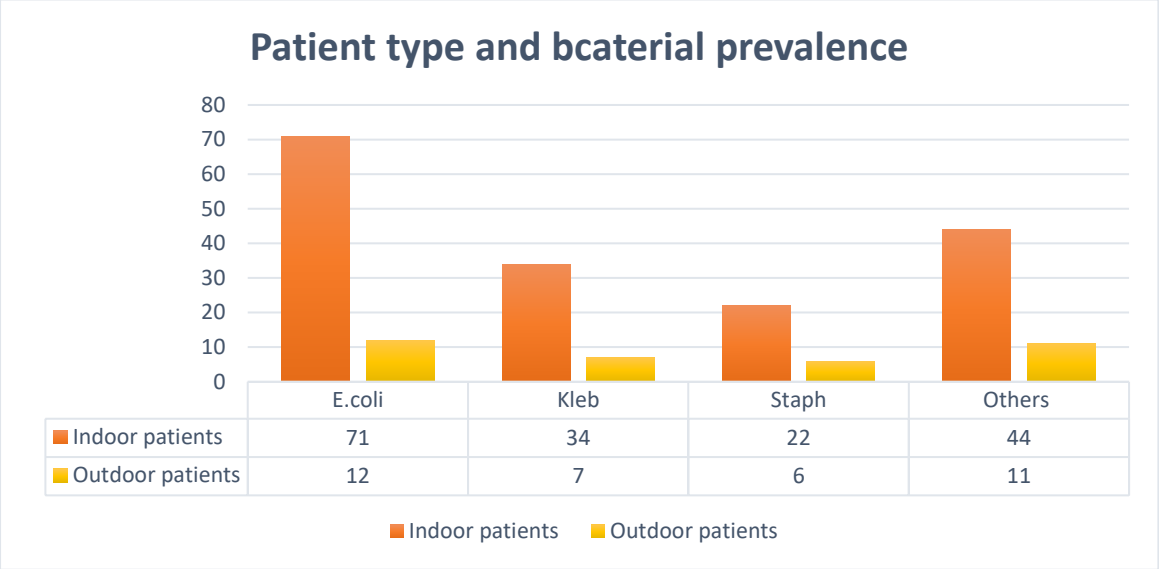

Figure 2 Bacterial prevalence in patients type

in total 207 positive samples number of gram negative was very much high which was 149 (72%) of total isolates with most prevalent *E.coli* and least prevalent was *Stenotrophomonas maltophilia*. If we compare Enterobacteriaceae with non-Enterobacteriaceae the number of Enterobacteriaceae was significantly high which is 124(83.2%) out of 149.

Antimicrobial sensitivity of all isolated Enterobacteriaceae were tested with antibiotics name as Ceftriaxone, Ceftazidime/avibactum, Imepenem, Meropenem, Amikacin, Tobramycin, ciprofloxacin and Co-trimoxazole. And percentage of their antibacterial susceptibility patterens and extended spectrum beta lactamases productivity are as followed.

Table 3 Antibiogram

Antibiotics	ICU	OPD	Orthopedic	Surgery	Pediatrics
ESBL +ve	95%	94%	91%	94%	88%
Imepenem	67%	88%	89%	72%	93%
Meropenem	72%	89%	89%	73%	94%
Amikacin	83%	94%	91%	80%	88%
Tobramycin	29%	41%	38%	21%	39%
Ciprofloxacin	8%	16%	7%	9%	19%
Ceftriaxone	5%	6%	9%	6%	12%
Ceftazidime/avibactum	88%	92%	88%	89%	93%
Co-trimoxazole	24%	31%	19%	23%	29%
Cefotaxime	5%	6%	7%	9%	19%

All isolated carbapenam resistant Enterobacteriaceae were further tested for carbapenam resistance genes, OXA-48, IPM, NDM, KPC and VIM by cartridge base PCR with Carb-R cartridge base

on real time PCR. In all CREs the prevalence of genes was as follows. Most prevalent gene in CRE was OXA-48 (98%) than IPM was (97.5%) than KPC (76%) and in last NDM which was 38%.

Discussion

The rise of ESBL-producing and CR Enterobacteriaceae isolates has significant clinical and therapeutic ramifications, which reduce the number of treatment choices available for individuals who are sick. In current study from 492 patients 11 hundred plus samples were collected and out of them the positive sample are 207, positivity rate is 17.9% positivity. Positivity on a maximum was in pus wound swab 88% and gram positive most prevalent organism, after blood was second most was blood and more precisely in ICU and surgery departments was 38%. The results of a retrospective study on 730 blood cultures taken from 718 patients were analyzed. The overall percentage of cultures that were positive came to 9.7 percent. Only 3.4% of the blood cultures were determined to have a real bacteremia, with the remaining samples being categorized as contaminants. Under 49 percent of the cases, the type of bacteria that was isolated was coagulase-negative staphylococci, which are regarded to be pollutants in any circumstance. 13.2 percent of all positive blood cultures were attributed to the presence of other pollutants (12). Enterobacteriaceae was most common pathogen in this study with *E.coli* on the top, in another study Carbapenem-resistant Enterobacteriaceae (CRE) infections represent a severe risk to patients in the hospital. Aside from their resistance to many other antibiotics, CRE can also be difficult to treat. Carbapenems will be replaced by few new antibiotics (13). Carbapenam resistance in ICU patient was highest 30% which is almost 7-8% high than other department isolates, because patient admitted to ICU are always at high risk to develop nosocomial infection of highly resistant strains (14). Which is also explained in a study conducted in India in which *K. pneumoniae* was the most frequent organism in our facility. Carbapenam resistant bacteremia is a late onset infection in patients exposed to antibiotics in the ICU and has a death rate of 60% at 30 days (15). In the present study carbapenam resistance genes *OXA-48, IPM, NDM, KPC,* and *VIM* were further amplified in all isolated carbapenam resistant Enterobacteriaceae using cartridge base PCR and Carb-R cartridge base on real time PCR. The frequency of genes was as follows in all CREs. OXA-48 was the gene with the highest prevalence in CRE (98%) followed by IPM (97.5%), KPC (76%) and last NDM (38%). While the majority of CRE clinical isolates from China were carbapenemases with traits similar to KPC-2, NDM, and OXA-48. The most common carbapenemase gene was *blaKPC-2* in isolates of *K. pneumoniae* from adult patients and *blaNDM* in isolates of E. coli from children (16). Bacterial pathogens have ability to change their genome to produce resistance mechanism against antibiotics, some of the pathogens can induce resistivity from other pathogen by Quorum sensing phenomena, identification of genotyping and phenotyping character of drug resistivity always help clinicians to manage infection by giving targeted and calculated dose of antibiotics.

REFERENCES

1. Firouzabadi D, Mahmoudi L. Knowledge, attitude, and practice of health care workers towards antibiotic resistance and antimicrobial stewardship programmes: A cross-sectional study. Journal of evaluation in clinical practice. 2020;26(1):190-6.
2. Ahmad M, Khan AU. Global economic impact of antibiotic resistance: a review. Journal of global antimicrobial resistance. 2019;19:313-6.
3. Lancellotti P, Musumeci L, Jacques N, Servais L, Goffin E, Pirotte B, et al. Antibacterial activity of ticagrelor in conventional antiplatelet dosages against antibiotic-resistant gram-positive bacteria. JAMA cardiology. 2019;4(6):596-9.
4. Avila-Novoa M-G, Solís-Velázquez O-A, Rangel-Lopez D-E, González-Gómez J-P, Guerrero-Medina P-J, Gutiérrez-Lomelí M. Biofilm formation and detection of fluoroquinolone-and carbapenem-resistant genes in multidrug-resistant Acinetobacter baumannii. Canadian Journal of Infectious Diseases and Medical Microbiology. 2019;2019.
5. KONG KF, Schneper L, Mathee K. Beta-lactam antibiotics: from antibiosis to resistance and bacteriology. Apmis. 2010;118(1):1-36.
6. Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. Infectious Diseases Society of America guidance on the treatment of extended-spectrum β-lactamase producing Enterobacterales (ESBL-E), carbapenem-resistant Enterobacterales (CRE), and Pseudomonas aeruginosa with difficult-to-treat resistance (DTR-P. aeruginosa). Clinical Infectious Diseases. 2021;72(7):e169-e83.
7. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. Infection Control & Hospital Epidemiology. 2008;29(11):996-1011.
8. Solomon SL, Oliver KB. Antibiotic resistance threats in the United States: stepping back from the brink. American family physician. 2014;89(12):938-41.
9. AMLADI AU, SUDARSANAM TD, KANDASAMY S, KEKRE N, VEERARAGHAVAN B, SAHNI RD. Evaluation of CHROMagar™ TMmSuperCARBA™ as a Phenotypic Test for Detection of Carbapenemase Producing Organisms. Journal of Clinical & Diagnostic Research. 2019;13(9).
10. Lavallée C, Rouleau D, Gaudreau C, Roger M, Tsimiklis C, Locas M-C, et al. Performance of an agar dilution method and a Vitek 2 card for detection of inducible clindamycin resistance in Staphylococcus spp. Journal of clinical microbiology. 2010;48(4):1354-7.
11. Decousser J-W, Poirel L, Desroches M, Jayol A, Denamur E, Nordmann P. Failure to detect carbapenem-resistant Escherichia coli producing OXA-48-like using the Xpert Carba-R assay®. Clinical Microbiology and Infection. 2015;21(2):e9-e10.
12. Stalnikowicz R, Block C. The yield of blood cultures in a department of emergency medicine. European Journal of Emergency Medicine. 2001;8(2):93-7.

13. Perez F, Van Duin D. Carbapenem-resistant Enterobacteriaceae: a menace to our most vulnerable patients. Cleveland Clinic journal of medicine. 2013;80(4):225.
14. Peabody MA, Van Rossum T, Lo R, Brinkman FS. Evaluation of shotgun metagenomics sequence classification methods using in silico and in vitro simulated communities. BMC bioinformatics. 2015;16(1):1-19.
15. Porwal R, Gopalakrishnan R, Rajesh NJ, Ramasubramanian V. Carbapenem resistant Gram-negative bacteremia in an Indian intensive care unit: a review of the clinical profile and treatment outcome of 50 patients. Indian Journal of Critical Care Medicine: Peer-reviewed, Official Publication of Indian Society of Critical Care Medicine. 2014;18(11):750.
16. Han R, Shi Q, Wu S, Yin D, Peng M, Dong D, et al. Dissemination of carbapenemases (KPC, NDM, OXA-48, IMP, and VIM) among carbapenem-resistant Enterobacteriaceae isolated from adult and children patients in China. Frontiers in Cellular and Infection Microbiology. 2020;10:314.