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Research Article

Frequency and Resistance Pattern of *Carbapenemases* (Class A & B) in *E. Coli* and *Klebsiella* Species

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Abstract: Background: *Carbapenems* are potent and strong antibiotics that are usually reserved as last resort for bacteria possessing basic antibiotic resistance. These are broad spectrum drugs with more effective coverage against gram negative organisms. The antibiotic resistance pattern observed in bacteria possessing chromosomal group 2f β-lactamases is unique. It involves resistance to *carbapenems* while remaining sensitive to broadly attacking cephalosporins.

Objective: This study aims to provide assessment of frequency of class A & B *carbapenamase* in *E.coli* and *Klebsiella* species producers and establish the resistance pattern from clinical isolates, at a tertiary care hospital in Karachi.

Materials and Methods: This is a cross sectional, prospective study conducted at general hospital liyari, Karachi during Jan 2022- Jan 2023. Sample size was calculated as 400. Samples were divided into Urine samples of urinary tract infection patients (n=150), Pus sample from different sites (n=100), Respiratory tract, tracheal aspirates and sputum sample (n=100) and blood sample from suspected septicemia (n=50). SPSS-22 was used to enter and analyze the data. Chi-square test was used to analyze significance between two mean values, keeping ≤0.05 as significant p-value.

Result: Distribution of microorganisms were reported as 120 (41.1%), 80 (28.5%), 2 (0.69%), 24 (8.36%), 55 (19.1%) and 6 (2%) of *E. coli*, *Klebsiella Pneumonia, K.Oxytoca*, Gram positive. Gram negative and Yeast respectively. The confirmed presence of *carbapenemase* (CP) was identifies in urine sample as 4/6 (26.6%) *E.coli* and 2/6 (13.3%) *Klebsiella*, while pus samples identifies 3/4 (20%) *E.coli* and 1/4 (6.6%) *Klebsiella*. Similarly samples of respiratory tract identifies 2/5 (13%) of *E.coli* and 3/5 (20%) of *Klebsiella*. The calculated p-value was insignificant with 0.834 and 0.913 for *E.coli* and *Klebsiella* respectively.

Conclusion: This study concludes that *E.coli* is by far the most frequently reported microorganism from class B while class A organisms are in lower frequencies. CRE indicated higher resistance from non-β-lactam antibiotics, limiting treatment options.

Keywords: Phenotypic, Detection, Resistance, *Carbapenemases*, *E. coli, Klebsiella*.

INTRODUCTION

With the increasing trend of self-diagnosis and internet surfing for medicinal purposes, the misuse of antibiotic has become a significant collateral damage, compounded by some physicians' desire to prescribe empirical and unnecessary antibiotics for 'effective' treatment. Some strong and deadly bacteria produce beta-lactamases that are enzymes that decompose the beta lactam ring contained in the penicillin group of antibiotics, rendering them ineffective and warranting the need of a beta lactamase inhibitor as a combination drug along with penicillin [1]. The causes and mutations that lead development of the beta lactamase enzyme and its types in bacteria is beyond the scope of the study. Beta Lactamase are further classified into Classes A, B, C and D, with A, B and D classes having enzymes exhibiting carbapenemase activity [2].

Carbapenems are potent and strong antibiotics that are usually reserved as last resort for bacteria possessing basic antibiotic resistance. These are broad spectrum drugs with more effective coverage against gram negative organisms. Meropenem and Imipenem are the conventional *carbapenems* with Doripenem and Ertapenem being the latest and more tolerable prototypes [3]. These carbapenemase enzyme containing organisms are called CRE or Carbapenem Resistant Enterobacteriaceae and mostly include gut residing organisms such as *Escherichia Coli*, *Salmonella*, and *Klebsiella* [4].

The antibiotic resistance pattern observed in bacteria possessing chromosomal group 2f β-lactamases is unique. It involves resistance to *carbapenems* while remaining sensitive to broadly attacking cephalosporin [5-8]. Due to the propagation of newfound adherents of proved carbapenemase families, it is now of added significance to seek to recognize the qualities of these enzymes, with all their depths and restrictions [6].

Our study will be focused on the frequency of *carbapenemases* in *Klebsiella* and *E. Coli* and their respective resistance patterns. *Carbapenems* resistant *Klebsiella pneumoniae* is proposed to

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cause resistance to *carbapenems* by two mechanisms [7]. First method includes the production of cephalosporinases along with compromised cell wall permeability that also causes hydrolysis of cephalosporin. Secondly, beta lactamase production causes the breakage of beta lactam ring in majority of antibiotics [9].

According to the Ambler classification, *carbapenemases* are divided into class A (*K. pneumoniae carbapenemase*, KPC), class B or metallo-β-lactamases (MBL) (such as New Delhi metallo-β-lactamases, NDM), and class D (OXA-48-like carbapenemases). Like other members of the MBL group, NDM uses zinc for the decomposition of the attacking antibiotics, and its action can be countered by Ethylenediaminetetraacetic Acid (EDTA), which works as a chelating drug [6, 9, 10]. KPC-producing *K. pneumoniae* (KPC-Kp) is a pathogenic strain with a marked adaptability potential, thereby causing higher resistance [7, 11].

This study aims to provide assessment of frequency of class A & B *carbapenamase* in *E.coli* and *Klebsiella* species producers and establish the resistance pattern from clinical isolates, at a tertiary care hospital in Karachi.

MATERIALS AND METHODS

This is a cross sectional, prospective study conducted at General Hospital Liyari, Karachi during January, 2022- January, 2023. After getting ethical approval from research review committee of institute (No. LGH/REC/163), a questionnaire was developed with the help of statistician and subject specialist. Sample size was calculated with the help of WHO sample size calculator, assuming 50% frequency and keeping confidence interval as 95% and margin of error as 5%, the minimum required sample size is 385. However, the patient flux was higher and 400 samples were collected.

Samples were divided into Urine samples of urinary tract infection patients (n=150), Pus sample from different sites (n=100), Respiratory tract, tracheal aspirates and sputum sample (n=100) and blood sample from suspected septicemia (n=50). The distribution was based upon frequency of sample collections. Samples were collected in sterilized containers with appropriate measurements by trained staff.

Patients with systematic or local infection admitted in different departments of hospital, all adult patients (15-80 years) of both genders were included. While multiple samples from same patients were excluded from the study. Performed biochemical test performed on specimen were Oxidase test, Citrate utilization test, Indole producing test, Urease test, Triple sugar iron test (TSI), Voges proskaver test and sugar fermentation test.

The results were recorded as recommended by Tsakris *et al*., zone diameter increase of ≥5 mm around Imipenem and meropenem discs containing EDTA were compared to Imipenem and meropenem without EDTA were considered positive for MβL.

STATISTICAL ANALYSIS

Statistical package of social sciences (SPSS) version 23 was used to enter and analyze the data. Continuous variable was analyzed with the help of mean and standard deviation, while frequency was measured for dependent variables such as gender, sample identification, test results etc. The paired sample t-test was used to assess the association between two variables. Chi-square test was used to analyze significance between two mean values, keeping ≤0.05 as significant p-value.

RESULT

Total 400 samples were included in the study, out of 400 samples 190 (47.5%) were collected from male patients while 210 (52.5%) were collected from female patients. Age was divided into categories, indicating 15 years gap reporting frequencies as 24 (6%) from 15-25 years, 32 (8%) from 26-35 years, 40 (10%) from 36-45 years, 84 (21%) from 46-55 years, 120 (30%) from 56-65 years and 100 (25%) from 66-80 years. Out of 400 samples, 280 (70%) samples were reportedly as positive while 120 (30%) reported as negative.

Distribution of microorganisms were reported as 120 (41.1%), 80 (28.5%), 2 (0.69%), 24 (8.36%), 55 (19.1%) and 6 (2%) of *E. coli*, *Klebsiella Pneumoniae*, *K. Oxytoca*, Gram positive. Gram negative and Yeast respectively (Fig. **1**).

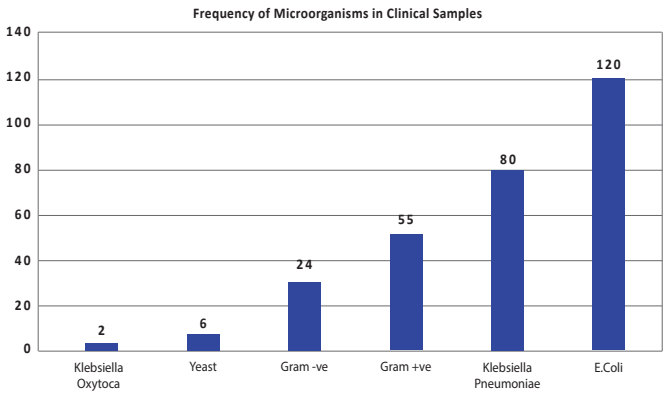

Fig. (1). Frequency of Microorganisms Identified in Clinical Samples of Study Participants.

Upon further distribution of microorganisms within collected sample of Urine, blood, respiratory site and pus the frequency was reportedly higher in urine samples with 80/130 (61.5%) of *E. coli* and 26/130 (20%) of *K. Pneumoniae* and 2/130 (1.5%) of *K. Oxytoca*, followed by Pus sample frequency of 30/84 (35.7%) of *E.coli* and 30/84 (35.7%) of *K. Pneumoniae*. Only significant p-value was identified in K.*Oxytoca* with 0.01, while *E. coli* had 0.71 and *K. Pneumoniae* had 0.09 p-value respectively (Table **1**).

Table 1. Association of Microorganism with Different Sample Types of Study Participants.

Organisms	Site of Sample Collection				P-Value
	Urine (n=130)	Pus (n=84)	Resp site (n=53)	Blood (n=13)	
E. Coli	80 (61.5%)	30 (35.7%)	6 (11.3%)	4 (30.7%)	0.713

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Klebsiella Pneumoniae	26 (20%)	30 (35.7%)	22 (37.7%)	2 (15.3%)	0.098
Klebsiella Oxytoca	2 (1.5%)	0	0	0	0.018

The confirmed presence of *carbapenamase* (CP) was identifies in urine sample as 4/6 (26.6%) *E.coli* and 2/6 (13.3%) and *Klebsiella*, while pus samples identifies 3/4 (20%) *E.coli* and 1/4 (6.6%) *Klebsiella*. Similarly, samples of respiratory tract identifies 2/5 (13%) of *E.coli* and 3/5 (20%) of *Klebsiella*. The calculated p-value was insignificant with 0.834 and 0.913 for *E.coli* and *Klebsiella* respectively (Table **2**).

Table 2. Distribution of Confirmed CP, *E. coli* & *Klebsiella* SPP in Clinical Specimen.

Organisms	Site of Sample Collection			P-Value
	Urine (n=06)	Pus (n=04)	Resp site (n=5)	
E. Coli	4 (26.6%)	3 (20%)	2 (13%)	0.834
Klebsiella Pneumoniae	2 (13.3%)	1 (6.6%)	3 (20%)	0.913

Confirmed CP was reported in 7/15 (46.6%) male participants and 8/15 (53.3%) of female participants, age categories identified as maximum frequency from 56-65 years group with 6/15 (40%), followed by 4/15 (26.6%).

Resistance pattern of various antibiotics were documented from study participants identified with *E.coli* and *klebsella* confirmation. The most frequently reported resistance was from Ampicillin with 116 (96.6%), followed by Cephalethin in 93 (77.5%) in *E. coli* group while the least reported resistance was from Tigecyclin in 3(2.5%) of patients. Similarly, the *Klebsiella* group reported higher resistance from Azotreonam with 82 (99.9%), and Ceftazidime in 82 (99.9%) followed by Cefotaxime, Ceftraxon and Amoxicillin with 81 (98.7%), 81 (98.7%),and 81 (98.7%), respectively (Table **3**).

DISCUSSION

Thorough research is being carried out at national and international levels for better understanding of causes of the ongoing resistance that has become a clinical challenge. Newer and stronger antibiotics are being introduced to tackle with the aforementioned scenario. Additionally, the origin of the resistance pattern at genetic level is being studied to create targeted therapies and genetically modified models.

Table 3. Antimicrobial Resistance Pattern of Isolated *E.coli* and *Klebsella*.

Antibiotics		Isolated Microorganisms	
		E.coli **(n=120)**	***Klebsiella*** **(n=82)**
Ampicillin	AMP	116 (96.6%)	71 (86.5%)
Amoxicillin-clavulanic acid	AMC	72 (60%)	81 (98.7%)
piperacillin/Tazobactam	TZP	24 (20%)	35 (42.6%)
Aztreonam	ATM	91 (75.8%)	82 (99.9%)
Cephalethin	CIP	93 (77.5%)	79 (96.3%)
Cefuroxime	CXM	83 (69.1%)	78 (95.1%)
Cefotaxime	CTX	74 (61.6%)	81 (98.7%)
Ceftazidime	CAZ	73 (60.8%)	82 (99.9%)
Ceftraxon	CRO	74 (61.6%)	81 (98.7%)
Cefepime	FEP	70 (58.3%)	76 (92.6%)
Ofloxacin	OFX	74 (61.6%)	47 (57.3%)
Ciprofloxacin	CIP	73 (60.8%)	45 (54.8%)
Gentamicin	CN	53 (44.1%)	59 (71.9%)
Amikacin	AK	11 (9.1%)	28 (34.1%)
Trimethoprim - Sulphamethoxazole	SXT	83 (69.1%)	82 (69.1%)
Imipenem	IPM	7 (5.8%)	9 (10.9%)
Meropenem	MEM	7 (5.8%)	9 (10.9%)
Tigecyclin	TGC	3 (2.5%)	4 (4.8%)

A study conducted at Karachi, Ziauddin Hospital, studied over 2000 samples, collected from urine, blood, pus, gastric, pleural, and tracheal sources, revealing a wide and versatile sample size [12]. Majority of the patients were of male gender. 60% of the samples showed *E.coli* while 22% were *Klebsiella*. Rest 10% were *Enterobacter* species. All of the isolates were sensitive

to Colistin, Tigecycline and Fosfomycin. A whopping 73.4% mortality rate was noted in the effected patients [13]. another Pakistani study presented a comprehensive examination of 178 *K. pneumoniae* isolates that produced extended-spectrum beta-lactamases (ESBLs), all of which were obtained from subjects residing in a distinctive area of Pakistan between 2010 and 2012 [14]. Within analysis, it was identified that there were two prominent lineages, yet the overarching resistance patterns and virulence-related elements did not account for their evolutionary success [15]. The resistance to these antibiotics could be attributed to *carbapenemases*, inhibitor-resistant beta-lactamase enzymes, or ampC-type beta-lactamases, with most, though not all, relevant strains featuring at least one of these factors [16]. It was also reported that complete genomes for six selected strains, offering detailed insights into the mobile genetic elements present during the initial dissemination of NDM-1. The unexplained success of specific lineages within this highly resistant strain pool, along with the disconnect between phenotypic resistance and genotype at a broader scale, suggests that inherent mechanisms play a role in conferring competitive advantage and/or resistance [9, 13, 15].

A study conducted in the Saudi Arabia in 2021 explored variable factors determining the resistance patterns in CREs. This study focused on *Klebsiella pneumonae* and the study spanned over a period of three years [17, 18]. Additionally, OXA-23 was detected in approximately 67% of all the isolates. The studies emphasized on the lethal potential of this *Klebsiella* specie to incur diverse clonal properties in terms of resistance patterns [10, 12, 15, 19].

Alizadeh N, *et al.* in 2022 published a two years study where they explores 60 samples and concluded the efficacy of Carba NP as an accurate test to detect carbapenemase enzymes. They concluded in favour of amikacin ax being the most effective treatment option against the lethal CRE as their isolates showed resistance to both imipenem and meropenem, detected via micro-broth dilution [11, 15, 18, 20].

In a 2020 Indian study conducted by Shenoy S, *et al*., published in the Journal of Krishna Institute of Medical Sciences, 51 multidrug-resistant *Klebsiella pneumoniae* strains were examined. These strains were collected from various clinical samples obtained from patients admitted to two different tertiary care hospitals over a 6-month period. Remarkably, 88.2% of the multidrug-resistant *Klebsiella pneumoniae* strains exhibited resistance to three or more classes of antimicrobial agents [12, 21].

The specimens yielded *Klebsiella pneumoniae* complex, *Escherichia coli*, and Enterobacter spp. Out of the 9,564 Enterobacterales isolated, 282 exhibited multidrug resistance (MDR). Notably, all these isolates were found to harbor carbapenemase-coding genes, with blaNDM (90%) and blaIMP (71%) being the most prevalent genes within the CRE strains. Among the isolates, 39.2% carried a combination of blaNDM- blaIMP, while 22.6% possessed a combination of blaNDM-blaIMP-bla-OXA-48-like genes [13, 22].

An Iranian study evaluated 1222 isolates of *Klebsiella*, *Enterobacter* and *E.coli* and after labelling them CRE against Meropenem and/or imipenem discs, they ran them against levofloxacin, rifampicin, Fosfomycin metronidazole, tigecycline and others. Levofloxacin came out as a promising alternative against Enterobacter species [14, 23].

Another study from Bahrain, imposed similar results with the emphasis on older age and ICU admissions as important risk factors for CRE acquisition. Tigecycline and colistin were found as best treatment options. This study spanned over a duration of 6 years, and was published in 2019 [15, 24].

Miftode IL, *et al.* evaluated organisms causing urinary tract infections. 354 samples were retrospectively analyzed and 25 patients were found were found to have CRE. Approximately 24% mortality was noted in CRE patients, other risk factors regarding hospitalization and patient's own conditions were also evaluated for mortality [16].

A recent study conducted by Madney Y, *et al*. in Egypt focused on the management of carbapenem-resistant Enterobacterales (CRE) infections in pediatric oncology patients. This retrospective investigation analyzed pediatric cancer patients who had experienced bloodstream infections (BSIs) caused by CRE at a children's cancer hospital in Egypt between 2013 and 2017. The study identified several key clinical factors contributing to the acquisition of CRE-BSI, including prior antibiotic exposure (90%), profound neutropenia (84%), extended use of steroids (45%), previous colonization with drug-resistant pathogens (35%), ICU admission within the preceding 90 days (28%), and the utilization of central venous catheters (24%). The primary pathogen isolated was *E. coli* (56%), shadowed by *Klebsiella pneumoniae* (37%), and all of these isolates displayed resistance to *carbapenems*. The study reported an overall mortality rate of 57%, with 30% of cases experiencing mortality within 30 days. Multivariate analysis revealed that patients with *Klebsiella pneumoniae* BSI, particularly those with carbapenem resistance with an MIC > 8 μg/mL and the presence of typhlitis or pneumonia, were at a higher risk of unfavorable outcomes. In conclusion, CRE-BSI poses a significant threat to pediatric cancer patients in resource-constrained countries, where treatment options are limited. The study underscores the importance of implementing antimicrobial stewardship practices, such as routine screening, prompt empirical treatment, and timely administration of appropriate therapy, to potentially improve the prognosis of this high-risk patient population [17].

Concluding with a Greek study by Dwomoh FP, *et al*. it should contrasting results as they recultured 144 isolates and ran them against a wide series of antibiotics, those MDR and CRE populations were found to have higher rates of resistance but were found to be low carbapenemase producers indicating other factors and genetic susceptibilities as a cause of the immaculate resistance pattern rather than the enzyme itself [18, 25].

CONCLUSION

This study concludes that *E.coli* is by far the most frequently reported microorganism from class B while class A organisms are in lower frequencies. CRE indicated higher resistance from non-β-lactam antibiotics, limiting treatment options. The increase in infection incidence caused by carbapenem can be handled with a uniform antibiotic policy and lowering the resistance.

AUTHORS' CONTRIBUTION

- **Hina Faisal**: Objective, write-up.
- **Amber Yasmeen Alvi**: Data Collection, Data entry.
- **Azra Idris**: Data Analysis, Laboratory work-up.
- **Maliha Yasmeen**: Write-up, Ethical considerations.
- **Naseha Mushtaq**: Write-up, results interpretation.
- **Hira Zafar Siddiqui**: Data entry, analysis and write-up.

CONFLICT OF INTEREST

Declared none.

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