Carbapenem Resistance among Enterobacteriacae, *Pseudomonas Aereuginosa* and *Acinetobacter Baumannii* isolated from Patients at Tertiary Care Hospitals of Peshawar

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ABSTRACT

Objective

To ascertain the incidence of Carbapenem resistant Enterobacteriaceae and to evaluate the pattern of antibiotics resistance for treatment of infections caused by these bacteria.

Methodology

This retrospective study was done in Microbiology department, Abasyn University Peshawar and Department of Microbiology, OK Quality Lab and Research Centre Peshawar, Pakistan. Data of 300 samples were studied from Medical Microbiology records of three tertiary care hospitals of Peshawar from November, 2020 to March, 2021.Microbial confirmation was done using API system of identification and antimicrobial resistance was determined by modified Kirby-bauer method. Detection of carbapenamase enzyme was done through Modified hodge test in this study.

Results

A total of 300 samples were studied. Those with a positive cultures, 71(49.3%) were males and 73(50.7%) were females. Out of these, 51(35.4%) cases were of UTI with positive urine culture and 39(27.1%) were diagnosed through pus culture. E.Coli was commonest microbe found while carbapenems had significant antibacterial activity. Modified Hodge test reveals that out of (n=46) only 8(17.4%) manifested phenotypic gene expression.

Conclusion

Our study identifies the emergence of Carbapenem resistant Enterobacteriaceae, *P.aeruginosa* and *A.baumannii* in local population. This situation is alarming, due to loss of options for the physicians in treating such multi-drug resistant infections. It is imperative to formulate a strategy for antimicrobial stewardship and control antimicrobial resistance in our country.

Key words: Antibiotic resistance, Carbapenems, Modified hodge test.

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INTRODUCTION

Resistance of infections reproduced by gram negative bacteria, *Enterobacteriaceae* has become a major health concern in some regions of the world. *Enterobacteriaceae* are gram negative bacteria found in gut of humans normally. They also make a part of normal flora. These bacteria can cause diseases if they colonize the gut heavily. Carbapenems have been utilized to treat the infections produced by *Enterobacteriaceae* for quite some time. Nowadays, Carbapenem resistance is getting prevalent in *Enterobacteriaceae.* It is the last option drug to treat infections caused by these bacteria.[1] The future of antibiotics is therefore dependent on Antimicrobial stewardship, which is a coordinated program that reduces further development of resistance by encouraging appropriate use of antimicrobials.[2]

Carbapenem are derivatives of thyanamycin and were developed in the 1980s. Carbapenems are β lactam antibiotics, as are penicillin and cephalosporin, but differ from these other generations in their exact chemical structure. Imipenem and meropenem, the first members of the type, had an extended spectrum of antibacterial activity that included coverage of *Pseudomonas aeruginosa,* endorsing it as a sole treatment for nosocomial infections. Back then, nearly all *Enterobacteriaceae* were nonresistant to Carbapenems.[3] A similar study conducted by Aga Khan University, Karachi from 2001-2006 indicated an increase in ESBL and multidrug-resistant organisms producing *Klebsiella pneumoniae* to >30% and 0.4% Carbapenem resistance. A study of blood stream infections from Lahore revealed an alarmingly high resistance in *Enterobacteriaceae* against 3rd

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generation Cephalosporins (93.7%); and 6.5% carbapenem resistance among *Pseudomonas* and *Acinetobacter* isolates. Moreover, infection with pan-drug resistant *Acinetobacter baumanii* is also increasing in many hospital settings across Pakistan with reported high mortality among patients.[4]

Resistance has been attributed mainly by production of β-lactamase enzymes by these *Enterobacteriaceae*. β-lactamase enzymes are classified as A,B,C and D. Resistance to carbapenem antibiotics is said to be due to: (a) Production of Carbapenemases (b) Poor binding to Penicillin binding proteins (c) Overexpression of multidrug efflux pumps and (d) Lack of porins in cell membrane of bacteria.[5] Carbapenemases producing *Enterobacteriaceae* or the Carbapenem resistant *Enterobacteriaceae* (CRE) have emerged, which confer broad resistance to most ß-lactam antibiotics including "last-line" Carbapenems. Another mechanism of resistance can be through porins deficiency, resulting in weakened entry of the ß-lactams through cell membranes, in combination with ESBLs.[6]

Resistance by these bacteria to antibiotics has arisen due to acquisition of carbapenamase genes.[7] Extended spectrum β-lactamases enzyme production by *Enterobacteriaceae* bacteria has resulted in increased resistance to cephalosporin antibiotics and due to tremendous usage of carbapenem antibiotics in extended spectrum β-lactamase endemic settings, emerging resistance to these drugs is now a major concern in many parts of the world.[8]

For diagnostic purposes, Carbapenemases gene detection by molecular methods is the benchmark but is available in only a few reference laboratories, and phenotypic tests have therefore evolved. Nowadays, for the purpose of Carbapenemases screening, the clinical and laboratory standards institute approves the modified Hodge test (CLSI., 2016).[9]

At present, in our country there is lack of data on resistance especially among *Enterobacteriaceae* to carbapenems. We undertook this study to determine the incidence of carbapenem resistant *Enterobacteriaceae* and to evaluate the pattern of antibiotics for treatment of infections caused by these microbes and also to identify the carbapenemases enzymes by Modified Hodge Test in *Enterobacteriaceae, P. aeruginosa* and *A.baumannii.*

METHODOLOGY

This is a retrospective study carried out in Department of Microbiology and Biotechnology, Abasyn University Peshawar and Department of Microbiology, Ok Quality Lab and Research Centre Peshawar, Pakistan. Study was approved by the Ethical committee, Abasyn University Peshawar. Data of 300 samples were studied from medical microbiology records and databases of Hayatabad Medical Complex (HMC), Lady Reading Hospital (LRH) and Khyber Teaching Hospital (KTH) Peshawar from November, 2020 to March, 2021 of patients of all ages and both sexes.

Samples included pus, blood, urine, cerebrospinal fluid (CSF), ascetic fluid, pleural fluid and wound swab. For isolation of gram-negative lactose fermenters from non-lactose fermenters, MacConkey's agar was used. The segregation of hemolytic from non-hemolytic colonies was obtained through blood agar plates and Chocolate agar was used as enriched media for the growth of fastidious bacteria. The Cysteine lactose Electrolyte Deficient (CLED) agar media which prevents overwhelming growth of *Proteus* mirabilis as well as differentiates gram negative non-lactose fermenters from lactose fermenters, was applied on Urine samples. Processing of blood samples was done using Tryptic Soya Broth (TSB), blood agar and MacConkey agar medium. Various Biochemical tests were performed for identifying *Enterobacteriaceae* family of bacteria and other gram negative non-fastidious rods.

Incubation of specimens at 37°C was done after their inoculation on blood and MacConkey's agar plates. API(biomerieux, France) system of identification was used to confirm the microbes. Modified Kirby-bauer method was applied on all the isolated microorganisms for determining antimicrobial susceptibility. CLSI 2016 criteria was followed for the interpretation of results. The gram-negative bacteria which were resistant to routine antibacterial drugs like Penicillins, cephalosporins Aminoglycosides, Macrolides, Tetracyclines and Quinolones and the gram-negative bacteria not having intrinsic resistance to carbapenems were taken in in this study. Patients with duplicate microbial isolates were excluded from this study.

Through the MHT test detection of carbapenamase enzymes in bacteria was done, when tested organism produces the carbapenamases and at same time allowing excrescence of an organism sensitive to carbapenem(*E.coli* ATCC 25922) towards the disk. Susceptibility of *E.coli* species to Carbapenems was judged through disk diffusion method using 10 µg Imipenem or meropenem susceptibility disk on MHA plates. For each strain, a suspension of bacteria adjusted to 0.5 McFarland turbidity standards was applied. Incubation of the plates was done for 16 to 24hours at 37◦C. CLSI 2016 criteria was used to interpret results. After the incubation, within the inhibition zone of the disk a clover-leaf like denting at junction of *E.Coli* 25922 and the test isolate was observed. A clover-leaf like indenting produced by E.Coli 25922 growing together with the test isolate growth streak inside the disk diffusion zone indicated a positive MHT.(CLSI guidelines, 2016).[9]

RESULTS

A total of 300 samples were collected and processed for isolation of bacterial pathogens. 144(48%) samples were positive for different pathogens while 156(52%) samples were negative. Those with a positive cultures, 71(49.3%) were males and 73(50.7%) were females. Out of these, 51(35.4%) cases were of UTI with positive urine culture, 39(27.1%) were diagnosed through pus culture, 21(14.6%) through blood cultures and 09(6.3%) had a wound swab positive (Figure 1).

Among the identified microorganisms; *E. Coli* was found in 43(29.9%) cases, Klebsiella in 32(22.2%) cases, *Pseudomonas aeuroginosa* in 23(16%) and *Salmonella typhi* in 07(4.9%) cases. *Proteus mirabilis* was identified in only 01(0.7%) cases. (Table 1)

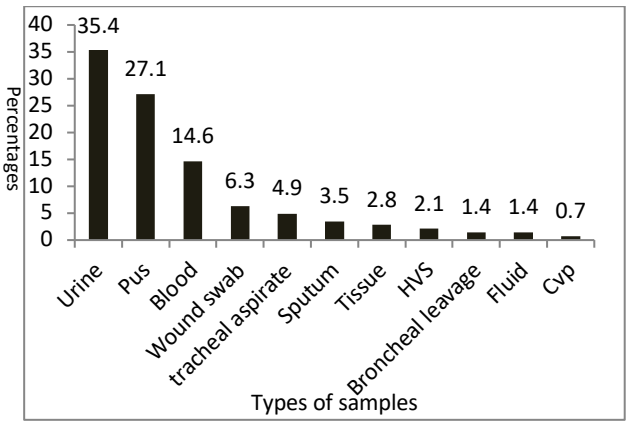

Figure 1: Frequency distribution and percentage of positive samples

S.No	Microorganism	Frequency	Percentage (%)
1	E.coli	43	29.9
2	Klebsiella species	32	22.2
3	P.aeruginosa	23	16.0
4	*A.baumannii*	18	12.5
5	P.vulgaris	14	9.7
6	S.typhi	07	4.9
7	C.freundii	04	2.8
8	Enterobacter species	01	0.7
9	M.morganii	01	0.7
10	P.mirabilis	01	0.7

Table 1 Frequency distribution of isolated microorganism

Most significant antibacterial activity was shown by Imipenem and Meropenem. Both had 68% sensitivity, followed by Tazobactum /piperacillin with 61.1% sensitivity and Ampicillin showed 14.6% sensitivity. The antibiotic Cotrimoxazole had only 38.2% sensitivity. (Table 2)

The result of Modified Hodge test (MHT) reveals that out of (n=46) only 8(17.4%) had shown phenotypic gene expression while 38 (82.6%) were negative. Among resistant strains, 09 (19.5%) were from *Klebsiella* species, whereas 05 (62.5%) of these developed resistant gene as detected by the modified Hodge test. (Table 3)

DISCUSSION

The administration of the right antibiotics can make a difference between cure and death of patient suffering from infection. Regrettably, due to misaligned perceptions, limitations in diagnosis and unrestricted consumption of antibiotics for years has resulted in evolution of resistant bacteria and got us to brink of post-antibiotic era (WHO 2015). In our study, 144(48%) samples were found positive for different bacterial pathogens while 156(52%) samples were negative. Culture & sensitivity analysis shows that 35.4% positive cases were detected through urine

	Antibiotics	Sensitive (%)	Resistant (%)
1.	Ampicilline	21(14.6%)	123(85.4%)
2.	Tazobactem pip	88(61.1%)	56(38.9%)
3.	Ceftazidime	56(38.9%)	88(61.1%)
4.	Ceftriaxone	52(36.1%)	92(63.9%)
5.	Cefixime	59(41.0%)	85(59.0%)
6.	Cefipime	62(43.1%)	82(56.9%)
7.	Aztreonam	56(38.9%)	88(61.1%)
8.	Imipenem	98(68.1%)	46(31.9%)
9.	Meropenem	98(68.1%)	46(31.9%)
10.	Gentamicin	74(50.7%)	70(48.6%)
11.	Amikacin	91(63.2%)	53(36.8%)
12.	Ofloxacilline	92(63.8%)	52(36.2%)
13.	Fosfomycin	56(38.9%)	08(5.6%)
14.	Nitrofurantoin	47(32.6%)	17(11.8%)
15.	Co Trimoxazole	55(38.2%)	88(61.1%)
16.	Cefoperazone/sulbactam	92(63.9%)	52(36.1%)

Table 2: Sensitivity pattern of Enterobacteriaceae, *P.aeruginosa* and *A.baumannii*

Bacterial species		Modified Hodge Test (%)		Total
		Positive	Negative	
1.	*Acinetobacter baumannii*	00(00.0%)	18(47.0%)	18 (39.1%)
2.	Citrobacter freundii	Nil	02(05.2%)	02(04.4%)
3.	Escherichia coli	01(12.5%)	02(05.2%)	03(06.5%)
4.	Klebsiella species	05(62.5%)	04(10.5%)	09(19.5%)
5.	Proteus vulgaris	02(25.0%)	01(02.6%)	03(06.5%)
6.	*Pseudomonas aeruginosa*	00(00.0%)	11(28.9%)	11(23.9%)
	Total	08(17.4%)	38(82.6%)	46(100%)

Table 3: Phenotypic detection of Carbapenem resistant Enterobacteriaceae, *P. aeruginosa* and *A.baumannii* isolates

samples, followed by pus 27.1%,blood 14.6%,wound swab 6.3% and sputum 2.5%.

Frequency of bacteria found in samples was; *E.Coli* 43(29.9%), *Klebsiella* 32(22.7%), *P.aereuginosa* 23(16%) and *A.baumanni* 12.5%. In another similar study done in Karachi city, E.coli was found to be the main pathogen in 71% cases, while second common was *Klebsiella* pneumonia & *Enterobacter* species.[12] *Pseudomonas aeruginosa* is a common nosocomial pathogen,

notorious for its multidrug resistance and life threatening infections in critically ill patients. Lately, Carbapenems are being used as the last resort antimicrobial to treat serious infections due to MDR *P.aeruginosa*. In a study, the rate of Carbapenem resistance in *P. aeruginosa* has been reported to vary from 12-37 percent.10 Health care acquired infections are caused by various bacteria, these bacteria becoming one of the high risk factor for the patient because of reducing compassion outline to antibacterial agents, while noticed in lactose non fermenting bacteria. In this group *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, have been found normally nearly in health care centers all over the world. The most common causative agent of nosocomial infection is *P. aeruginosa* which shows multidrug resistance and is threat to increase mortality rate and limit the choice of drug to treat severe infections due multidrug resistant *P. aeruginosa*. *Pseudomonas* species were (50%) resistant to gentamicin, chloramphenicol (100%), amoxicillin/clavulanic acid (100%), ampicillin (100%) and ceftriaxone (100%). All Proteus and *Pseudomonas* isolates were susceptible to ciprofloxacin.[10]

There were 18 (39.1%) resistant cases of *A.baumannii* but not expressing any phenotypical gene expression. Bayram *et al* . also described the resistance of *Acinetobacter* species to most of the antibiotics tested. Sensitivity was shown to gentamycin, meropenem and amikacin. *Acinetobacter* strains are often resistant to antimicrobial agents.[11] During our study the Proteus vulgaris and *Pseudomonas aeruginosa* accounted for 3(6.5%) and 11(23.9%) of resistant group of microorganism, whereas 02 (25.0%) of Proteus vulgaris showed phenotypical expression of resistant gene however there was no such expression of resistant gene among *P.aeruginosa* species. A study was also conducted by Muthusamy *et al* . on multidrug resistance and depicted that *A.baumannii* has emerged as an important and problematic human pathogen as it is the causative agent of several types of infections. *A.baumannii* causes a wide range of infections such as ventilator associated pneumonia, septicemia, urinary tract infections, wound infections, and meningitis especially in immune compromised and hospitalized patients.[12]

In present study, significant antibacterial sensitivity was shown by Imipenem 68%, Piperacillin 61.1% and Ampicillin 14.6%. Mos *et al* . also reported in his study that sensitivity to amikacin was 75%, Imipenem 52.3% and it was between 35-50% to fourth generation of antibiotics Cephalosporins. The lowest sensitivity was noted for Ampicillins i.e, 6.8%.[13] The sensitivity to penicillins & cephalosporins was less, with the number of resistant bacterial strains being above 50% in contrast to 19.5% observed in the Sentry programme in 2004. While looking into the antimicrobial susceptibility of the bacteria, Imipenem & Meropenem antibiotics were showing Sensitivity in 98(68%) of the samples and Resistance to 46(31.9%) of the samples. According to Rao *et al* ., a high degree of sensitivity to Linezolid, Vancomycin, Chloramphenicol and teicoplanin was observed in case of gram positive cocci while extended sensitivity to imipenem, aminoglycosides and piperacillin/tazobactum was observed for gram negative bacilli.[14]

The antibiogram of gram positive and gram negative bacteria as reported by Dessalegn *et al* ., revealed sensitivity to vancomycin in case of gram positive cocci and to imipenem by the gram negative bacteria. Sensitivity to all of the antibiotics tested was not observed in any of the bacterial isolates.[15] Timely identification of carbapenem strains is pivotal for controlling these infections in hospital settings. A previous study reported that 43.5% of antimicrobials were inappropriately prescribed; of these, broad-spectrum activity (44.6%) and use of antimicrobials without culture (32.4%) were the main reasons.[16] Inappropriate prescriptions when coupled with self-medication and improper antibiotic consumption may greatly enhance antimicrobial resistance. Appropriate antibiotic prescription guidelines, regulation of antibiotic dispensing by community pharmacies, and patient education are some of the vital measures in combating the menace of antimicrobial resistance.[16]

In this study Modified hodge test was used as gold standard for phenotypic detection of *Pseudomonas aeruginosa*, A. *baumanii* and Enterobacteriaceae resistant to carbapenems. It revealed that there was no gene expression in P.aeruginosa, C.freundii and A. *baumanii* although these bacteria were not susceptible to meropenem and imipenem antibiotics. Resistant cases of *Acinetobacter baumannii* were 18(39.1%) but phenotypic gene expression was not observed in them. Moreover, out of the resistant bacterial strains 6.5% were E.Coli while 12.5% of them expressed a positive modified hodge test. Additionally, among resistant strains, klebsiella species accounted for 19.5% although 62.5% of these developed resistant gene. In a similar study by Bayram *et al* ., *Acinetobacter baumanii* species showed greatest resistance to the antibiotics tested. In contrast there was high sensitivity to meropenem and gentamycin.[11] Due to the high degree of resistance expressed by *Acinetobacter baumanii* strains treatment can be really challenging. In sharp contradiction to our study, Bayram *et al* . in their research at Turkey had concluded that *Acinetobacter baumanii* were least susceptible to imipenem, meropenem, ceftazidime, cefipime, piperacillin/tazobactum, and gentamicin antibiotics.[11]

For better infection control in all of the DHQ (District Headquarter) Hospitals in KPK, similar studies are needed to give a better view of gram negative bacterial pathogens and their antibiotics resistance pattern. Inappropriate prescriptions along with self-medication and improper antibiotic consumption also needs to be discouraged in society. Molecular level studies are required for genotyping to detect the carbapenemases i.e. KPC, NDM, OXA and IMP etc. genes which are responsible for multi-drug resistance.

CONCLUSION

This study revealed that the isolates of *Enterobacteriaceae, A.baumannii and P.Aeruginosa* which are resistant to carbapenems, exhibited multi-drug resistance characteristics as assessed in local population at the three big tertiary care hospitals of Peshawar. In laboratory, through the method of phenotypic detection of meropenem and imipenem resistance in gram-negative bacterial isolates, it was reported to be 31.9%.

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