

## Multidrug resistance in Gram-negative pathogens isolated from patients with chronic kidney diseases and renal transplant

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### Abstract

Multidrug resistance (MDR) in gram-negative pathogens is the emerging threat to clinicians. The current study was designed to determine the prevalence and pattern of multidrug resistance in gram-negative clinical isolates. It was conducted at the COMSATS Institute of Information Technology, Islamabad, Pakistan, from June to October 2014. Of the 8,300 samples collected, 729(8.8%) clinically important gram-negative pathogens were retrieved. These pathogens were subjected to phenotypic and biochemical detection and were further processed for multidrug resistance pattern. It was observed that gram-negative pathogens were simultaneously resistant to many antibiotics. The prevalence of extended spectrum  $\beta$ -lactamase phenomenon was 220(100%) in *Klebsiella pneumoniae*, 195(75%) in *Escherichia coli*. Resistance to carbapenem was 174(79%) in *Klebsiella pneumoniae* and 14(5.4%) in *Escherichia coli*. Resistance against fluoroquinolones also displayed an escalating trend. The current study found that resistance against antibiotics was displaying a drastic increase in chronic renal patients.

**Keywords:** Extended spectrum  $\beta$ -lactamase, Antibiotics, Multidrug-resistant pathogens, *Klebsiella pneumoniae*, *Escherichia coli*.

### Introduction

In late 20th century, resistance in gram-positive pathogens was displaying an alarming situation in the healthcare system. However, with control policies and development of new antibiotics, it is under good control now. But the situation against gram-negative pathogens has aggravated to such an extent that once neglected class of antibiotic (polymyxins) is in use. The resistance in gram-negative pathogens has invalidated all the available therapeutic options and there is no hope to have any new class of antibiotics in the near future. Colistin or polymyxin E was thought to be toxic for humans, but the current situation has raised its usage as a last treatment

option to cure infections caused by multi-drug resistant (MDR) gram-negative pathogens.<sup>1</sup>

Enterobacteriaceae are a large class of bacteria and most of the normal gastrointestinal (GI) flora are also included in it. Yet, many clinically important pathogens belong to this class, too. These pathogens usually cause gastrointestinal tract (GIT) infections, particularly diarrhoea. Most of the infections caused by them exhibit spontaneous recovery. Nonetheless, studies from the past few years are demonstrating an intimidating situation. Ultimately, diarrhoea would be difficult to treat because of the emergence of resistance to the treatment of last resort.<sup>2</sup>

Many resistance mechanisms are described in enterobacteriaceae. Notable is the production of enzymes (beta [ $\beta$ ]-lactamases) that cleave the  $\beta$ -lactam ring in the whole range of  $\beta$ -lactam antibiotics. A large variety of  $\beta$ -lactamases are available; however, the most significant from clinical perspective are extended spectrum beta lactamases (ESBL), oxacillinase, chromosomally encoded class C cephalosporinases (AmpC) and carbapenemases.<sup>3,4</sup>

Most of these resistance genes in enterobacteriaceae are located on plasmids and thus easily spread between different species and across the genera. Infections caused by these MDR pathogens can only be treated with combination therapy and higher antibiotic doses and hence it is associated with numerous side effects. Therapeutic choices are limited to fosfomycin and colistin only.<sup>5</sup> Local epidemiology efforts can provide detection and timely response to MDR outbreaks. Surveillance studies can augment these efforts by recognising slower long-term resistance trends. Together, the information from these studies can support infection control interventions and antibiotic stewardship programmes.

As chronic renal failure is always concomitant with immune system deficiencies, patients undergoing dialysis have an amplified threat for getting a healthcare-associated infection (HAI), too, because of the frequent use of urinary catheters, needles and injections. Bacterial infections represent a common and important health problem for patients with end-stage renal disease (ESRD) and in those

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who undergo maintenance haemodialysis. All these patients illustrate the challenges inherent to this problem.<sup>6</sup>

The current study was planned to explore the prevalence of resistance in gram-negative pathogens isolated from chronic kidney diseases and renal transplant patients.

## Methods and Results

This cross-sectional study was conducted at the Department of Biosciences, COMSATS Institute of Information Technology (IIT), Islamabad, Pakistan, from June to October 2014, in collaboration with Al-Sayed Hospital, Rawalpindi, Pakistan. Most of the patients from whom the samples were taken were with chronic renal failure and kidney transplant. Hence this study was unique as almost all the patients were immunocompromised. In immunocompromised patients, it is significant to choose antibiotic therapy with caution as the resistant pathogens are very difficult to treat.

Routine specimens, including pus swabs, nasal swabs, throat swabs, urine samples, catheter tips, sputum, etc. were included in this study. All specimens were inoculated on appropriate culture media (cysteine-, lactose- and electrolyte-deficient [CLED] media for urine

culture/sensitivity [C/S], blood agar and MacConkey agar for pus swabs, nasal swabs and catheter tips. Blood and chocolate agar for sputum and throat swab) and incubated at 37°C for 24 hours. A total of 8,300 samples were collected and processed during the study period. Microorganisms were identified by standard microbiological procedures, i.e. gram staining, colony morphology, catalase test, motility, oxidase test via analytical profile index (API) 20E kits. For the purpose of this study, we collected aerobic, gram-negative rods (including enterobacteriaceae, pseudomonas (P.) aeruginosa, acinetobacter (A.) baumannii and burkholderia (B.) cepacia). They were isolated and their percentage was calculated. Individual frequencies of different organisms were also found. They were subjected to antimicrobial sensitivity testing as per guidelines of the Clinical and Laboratory Standards Institute (CLSI).<sup>7</sup>

Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method. Turbidity of the bacterial inoculum was compared with 0.5 McFarland turbidity standard and then inoculation was done with the help of sterilised cotton swabs on Mueller-Hinton (MH) agar. Antibiotic discs including ampicillin (AMP 10 µg), augmentin (AMC 30 µg), doxycycline (DO 30 µg), levofloxacin (LEV 5 µg),

**Table:** Prevalence of resistance in Gram negative pathogens isolated from chronic renal failure patients.

	K. pneumoniae n=220	A.baumannii n=23	P. aeruginosa n=143	M. morganii n=28	E.coli n=257
Ampicillin (AMP)	220	23		28	252
Augmentin (AMC)	220	23		28	234
Doxycycline (DO)	40	No			51
Amikacin (AK)	85	5	72	NO	27
Ciprofloxacin (CIP)	111	5	72	10	196
Enoxacin (ENO)	111	5	72	10	197
Levofloxacin (LEV)	111	5	72	10	192
Cefoperazone (CFP)	220	5	77	10	195
Cefotaxime (CTX)	220	5		10	195
Ceftriaxone (CRO)	220	5		10	195
Cefepime (FEP)	220	5	72	10	195
Meropenem (MEM)	174	5	30	NO	14
Imipenem (IPM)	174	5	22	NO	14
Trimethoprim/sulfamethoxazole (SXT)	220	5		28	209
Cefoperazone-Sulbactam (SCF)	149	5	42	10	139
Piperacillin-tazobactam (TZP)	179	5	42	10	145
Piperacillin- Sulbactam (SPR)	179	5	42	10	135
Fosfomycin (FOS)*	40/100	5	NO	28	10/110
Colistin (CT)	NO	NO	NO	28	NO
Ceftazidime (CAZ)	179	5	62	10	195
Tigecycline (TGC)	40	NO		8	33
Nitrofurantoin* (NF)	70/100	23		20	30/110

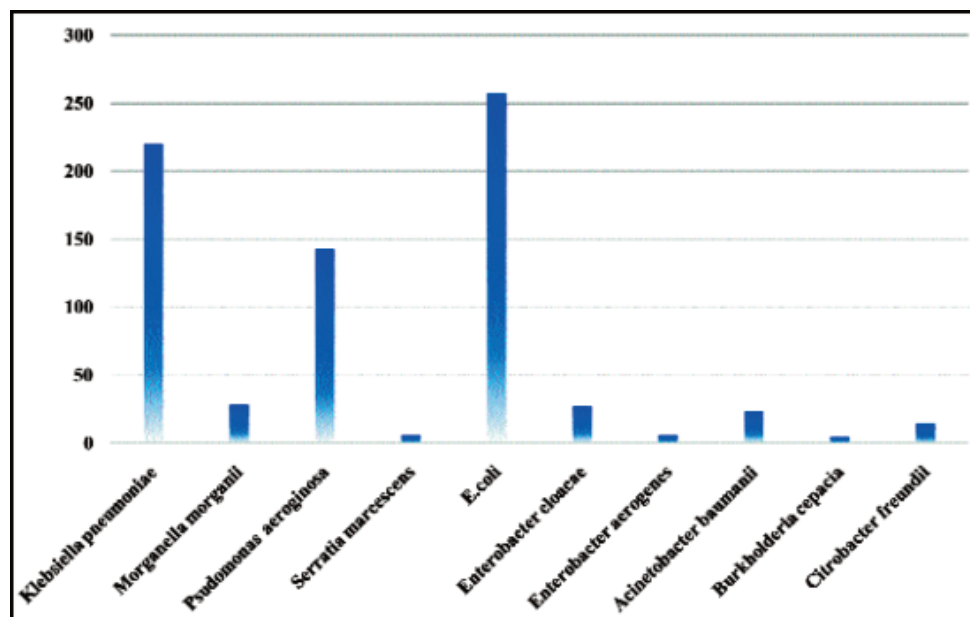
\*Nitrofurantoin and Fosfomycin was applied only on urine samples.

M. morganii: Morganellamorganii

P. aeruginosa: Pseudomonasaeruginosa

K. pneumoniae: Klebsiellapneumoniae

A. baumannii: Acinetobacterbaumannii.°



E. coli: *Escherichia coli*

**Figure:** Distribution of pathogens among isolated Gram negative pathogens.

gentamicin (10 µg), colistin (CT 10 µg), meropenem (MEM 10 µg), imipenem (IPM 10 µg), amikacin (AK 30 µg), piperacillin-tazobactam (TZP 110 µg), cefoperazone-sulbactam (SCF 105 µg), aztreonam (30 µg), polymixin-B (300 units), cefoperazone (CFP 75 µg), cefotaxime (CTX 30 µg), ceftazidime (CAZ 30 µg), ceftriaxone (CRO 30 µg), cefepime (FEP 30 µg), trimethoprim/sulfamethoxazole combination commonly known as co-trimoxazole (SXT 25µg), ciprofloxacin (CIP 5 µg), nitrofurantoin (NF 300 µg), fosfomycin (FOS 50 µg), tigecycline (TGC 15 µg) and tobramycin (10 µg) were applied on the plates. The plates were then incubated at 37°C for 24 hours and the results were interpreted according to the CLSI guidelines.<sup>7</sup>

ESBL was detected by double-disc diffusion test. An amoxyclav (amoxicillin/clavulonic acid 30/10 µg) disc was placed in the middle of petriplate and third-generation cephalosporin discs were placed 20-30mm away from the central disc. An extension in the zone of inhibition, so-called keyhole effect, was considered as positive for ESBL production.<sup>8</sup>

Carbapenem resistance was measured by susceptibility profile of imipenem and meropenem discs, i.e. zone less than 25mm was considered resistant.<sup>7</sup>

Data was entered in and analysed using Microsoft Excel 2010.

Of the 8,300 samples processed, 729(8.8%) samples of gram-negative microbes were isolated. Of them, *Escherichia (E.)coli*

represented the highest number 257(35%), followed by *Klebsiella (K.) pneumoniae* 220(30%) (Figure).

The ESBL phenomenon was detected in 438(60%) samples. All the *K. pneumoniae* samples were found to be highly resistant to cephalosporins. ESBL was prevalent in all the *K. Pneumonia* samples. In case of *E.coli*, ESBL was present in 195(75%) samples. *Enterobacter cloacae* was retrieved in 27(3.7%) samples and ESBL were isolated in 270(37%) samples. *Enterobacter aerogenes* was detected in just 6(0.8%) samples and only 1(0.14%) was ESBL positive.

The carbapenem resistance was observed in 174(79%) of *K. pneumoniae* population, 5(21%) in *Acinetobacterbaumanii*, 30(21%) in *P. aeruginosa* and 14(5.4%) in *E.coli*. In *Morganellamorganii*, all samples were susceptible to carbapenems.

In case of *K. pneumoniae*, resistance against fluoroquinolones was almost 111(50%), 5(21%) in *Acinetobacterbaumanii*, 72(50%) in *P.aeruginosa*, 10(35%) in *Morganellamorganii* and 197(77%) in case of *E.coli*.

Resistance against tigecycline was recorded to be 40(18%) in case of *K. pneumoniae* and 33(12.8%) in case of *E. coli* (Table).

## Discussion and Conclusion

Of the Gram-negative pathogens, *E. coli* comprised the majority of isolates (35%), followed by *K. pneumoniae* (30%) and *P.aeruginosa* (19.6%). The pattern of prevalence observed in this study was quite similar to previously reported single maintenance and reliever therapy (SMART) studies from the United States and North America. Accordingly, *E. Coli* comprised the majority of isolates, followed by *K. pneumoniae*, *P.aeruginosa*, and *Enterobacter* species.<sup>9</sup>

*Pseudomonas aeruginosa* is frequently present Gram-negative pathogens causing symptoms in immunocompromised patients, particularly in hospitalised patients. *P.aeruginosa* has the tendency to develop antibiotic resistance very rapidly. In a previous study, out of

the 3,700 samples, 102(2.7%) were identified as multi-drug resistant *Pseudomonas* (MDRP) *aeruginosa*. Whereas in the current study, it was recorded to be 1.7% of the total population.<sup>10</sup>

Sader et al. already provided evidence of rising bacterial resistance over time; their trend analysis showed an increase in the rate of ESBL-positive *Klebsiella* spp. in European intensive care units (ICUs) from 27.5% in 2009 to 41.8% in 2011.<sup>11</sup> However, in the current study, ESBL prevalence was monitored to be dramatically high. Moreover, 100% ESBL prevalence in case of *Klebsiella* spp. was observed whereas 75% in case of *E. coli*.

Even more worrisome than the increase in ESBLs is the concurrent rise in carbapenemase-positive *K. pneumoniae*. In a recent study, it was recorded to be increased from 9.3% to 18.3% in European ICUs. Similarly, European Antimicrobial Resistance Surveillance Network (EARS-Net) also reported a significantly increasing trend in carbapenem resistance in *K. pneumoniae* in Europe from 3.2% in 2009 to 6.2% in 2012.<sup>12</sup> In the present study, carbapenem resistance was recorded in ESBL positive *K. pneumoniae*, contributing to 79%, whereas in case of *E. coli* it contributed to almost 5%.

In many other studies, resistance against carbapenems has been reported more than 50% and carbapenem resistant pathogens were found to be resistant to all other treatment except tigecycline or colistin. Therefore, tigecycline is now considered as a last resort drug against MDR Enterobacteriaceae.<sup>13</sup> In this study, 100% of *K. pneumoniae* were found to be susceptible to colistin. However, 100% resistance against colistin was noted in case of *Morganella morganii*. Nonetheless, colistin is a reasonable safe last-line therapeutic alternative for MDR Gram-negative pathogens.<sup>1</sup> As far as tigecycline is concerned, resistance is displaying an emerging trend where 10-20% resistance was observed.

Our results of resistance to fluoroquinolone were also in good accordance to previous studies. In the present study, 50% of *K. pneumoniae* isolates and 74% of ESBL-positive *E. coli* were resistant to fluoroquinolones. Previously, 18% and 12.5% of the ESBL-positive *E. coli* and *K. pneumoniae* isolates were reported to be susceptible to levofloxacin, respectively.<sup>6</sup>

*E. coli* was found to be the most prevailing microbes amongst all Gram-negative pathogens in the current study, followed by *K. pneumoniae*. ESBL phenomenon and carbapenem resistance displayed haunting escalation in *K. pneumoniae*.

The most effective therapeutic regime was colistin for curing these MDR pathogens. Knowledge of the epidemiology of MDR pathogens, their resistance trends, and susceptibility patterns are useful to influence evolving guidelines for empiric therapy of MDR Gram-negative pathogens.

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