






## RESEARCH ARTICLE

# REVISED Molecular Epidemiological Surveillance of CTX-M-15-producing *Klebsiella pneumoniae* from the patients of a teaching hospital in Sindh, Pakistan. [version 3; peer review: 3 approved]

Previously titled: Molecular Epidemiological Surveillance of CTX-M-15-producing *Klebsiella pneumoniae* from the patients of a teaching hospital.

Muzaheed Muzaheed <sup>1</sup>, Naveed Sattar Shaikh<sup>2</sup>, Saeed Sattar Shaikh <sup>1</sup>, Sadananda Acharya<sup>3</sup>, Shajiya Sarwar Moosa<sup>4</sup>, Mohammad Habeeb Shaikh <sup>5</sup>, Faisal M. Alzahrani<sup>1</sup>, Amer Ibrahim Alomar<sup>1</sup>

<sup>1</sup>Department of Clinical Laboratory Science, College of Applied Medical Sciences, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

<sup>2</sup>Department of Medicine, People's University of Medical and Health Sciences, Nawabshah, Pakistan

<sup>3</sup>Department of Public Health, College of Public Health, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

<sup>4</sup>Department of Anatomy, College of Medicine, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

<sup>5</sup>College of Medicine, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

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## Abstract

### Background

The presence of Extended-spectrum  $\beta$ -lactamase positive bacteria in hospital setting is an aggravating influential factor for hospitalized patients, and its consequences may be hazardous. Therefore, there is a need for rapid detection methods for newly emerging drug-resistant bacteria. This study was aimed at the molecular characterization of Extended-spectrum  $\beta$ -lactamase -positive *Klebsiella pneumoniae* isolates recovered from the patients of a teaching hospital in Sindh, Pakistan.






### Methods


A total of 513 *K. pneumoniae* isolates were obtained from various clinical samples during June 2019 to May 2020. The collected isolates were investigated for antimicrobial susceptibility (antibiogram), and PCR and DNA sequencing were performed to analyse the ESBL genes.

### Results

## Open Peer Review

Reviewer Status 

	Invited Reviewers		
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1. SUPRIT DESHPANDE , Translational Health Science & Technology Institute, Faridabad, India


Among the 513 isolates, as many as 359 (69.9%) were Extended-spectrum  $\beta$ -lactamase producers and 87.5% were multi-drug resistant, while none had resistance to imipenem. PCR scored 3% blaTEM, 3% blaSHV, and 60% blaCTX-M-15 genes for the tested isolates.

### Conclusion

The study showed that CTX-M-15 was the major prevalent Extended-spectrum  $\beta$ -lactamase type among the isolates. Additionally, all the isolates were susceptible to carbapenems. Screening and detection of Extended-spectrum  $\beta$ -lactamase tests are necessary among all isolates from the enterobacteriaceae family in routine microbiology laboratory to prevent associated nosocomial infections. A larger study is essential to understand molecular epidemiology of Extended-spectrum  $\beta$ -lactamase producing organisms to minimize morbidities due to these multidrug resistant organisms.

### Keywords

K. Pneumoniae, ESBL, TEM, SHV, CTX-M-15

2. **Abbas Farahani** , Hormozgan University of Medical Sciences, Bandar Abbas, Iran

3. **Mubashir Zafar**, Aga Khan University, Karachi, Pakistan

Any reports and responses or comments on the article can be found at the end of the article.

**Corresponding author:** Saeed Sattar Shaikh ([ssshaikh@iau.edu.sa](mailto:ssshaikh@iau.edu.sa))

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**REVISED Amendments from Version 2**

In response to reviewers comments the following updates were done:

Updated Title: Molecular Epidemiological Surveillance of CTX-M-15-producing *Klebsiella pneumoniae* from the patients of a teaching hospital in Sindh, Pakistan.

Reason for update: As suggested by reviewer.

Abstract: Updated the text: This study was aimed at the molecular characterization of Extended-spectrum  $\beta$ -lactamase-positive *Klebsiella pneumoniae* isolates recovered from the patients of a teaching hospital in Sindh, Pakistan.

Reason for update: Clearfield the objective.

Discussion: Updated text: Larger studies need to be done in various geographical regions of the country to better define the molecular epidemiology of ESBL-producing *K. pneumoniae* and its clinical implications.

Reason for update: Provided details about limitations of the study.

References: Updated text: Naeem S, Bilal H, Muhammad H, Khan MA, Hameed F, Bahadur S, & Rehman TU. Detection of blaNDM-1 gene in ESBL producing Escherichia coli and Klebsiella pneumoniae isolated from urine samples. *J. Infect. Dev. Ctries.* 2021; **15**(4), 516–22. <https://doi.org/10.3855/jidc.12850>

Reason for update: Included recent relevant reference added.

**Any further responses from the reviewers can be found at the end of the article**

## Introduction

The emergence of Extended-spectrum  $\beta$ -lactamase (ESBLs) producing organisms has imposed a great threat on majority of the antibiotic classes, particularly cephalosporins.<sup>1</sup> The situation worsens when the patient infected with ESBL-producing organism is administered an antibiotic to which the organism is resistant.<sup>1</sup> Clinical practitioners globally have been facing problems related to plasmid mediated ESBL producing *Klebsiella pneumoniae* (*K. pneumoinae*). These plasmids contain genes encoding resistance to antibiotics such as aminoglycosides, sulfonamides, tetracycline, chloramphenicol, and quinolones.<sup>2</sup> Cephalosporins are  $\beta$ -lactam antibiotics that are widely used in clinical practice and in the treatment of bacterial infections.<sup>3</sup> In addition, *K. pneumoniae* is reported in nosocomial infections and community acquired infections.<sup>4</sup>

A study conducted by Maltezou et al.<sup>5</sup> revealed that the incidence rate of ESBL producing *K. pneumoniae* infection was 16.71% for Northern Europe, 24.41% for Southern Europe, 58.71% for Eastern Europe, 28.2% for the Asia Pacific region, 51.9% for South America, and 12.3% for North America.<sup>5</sup>

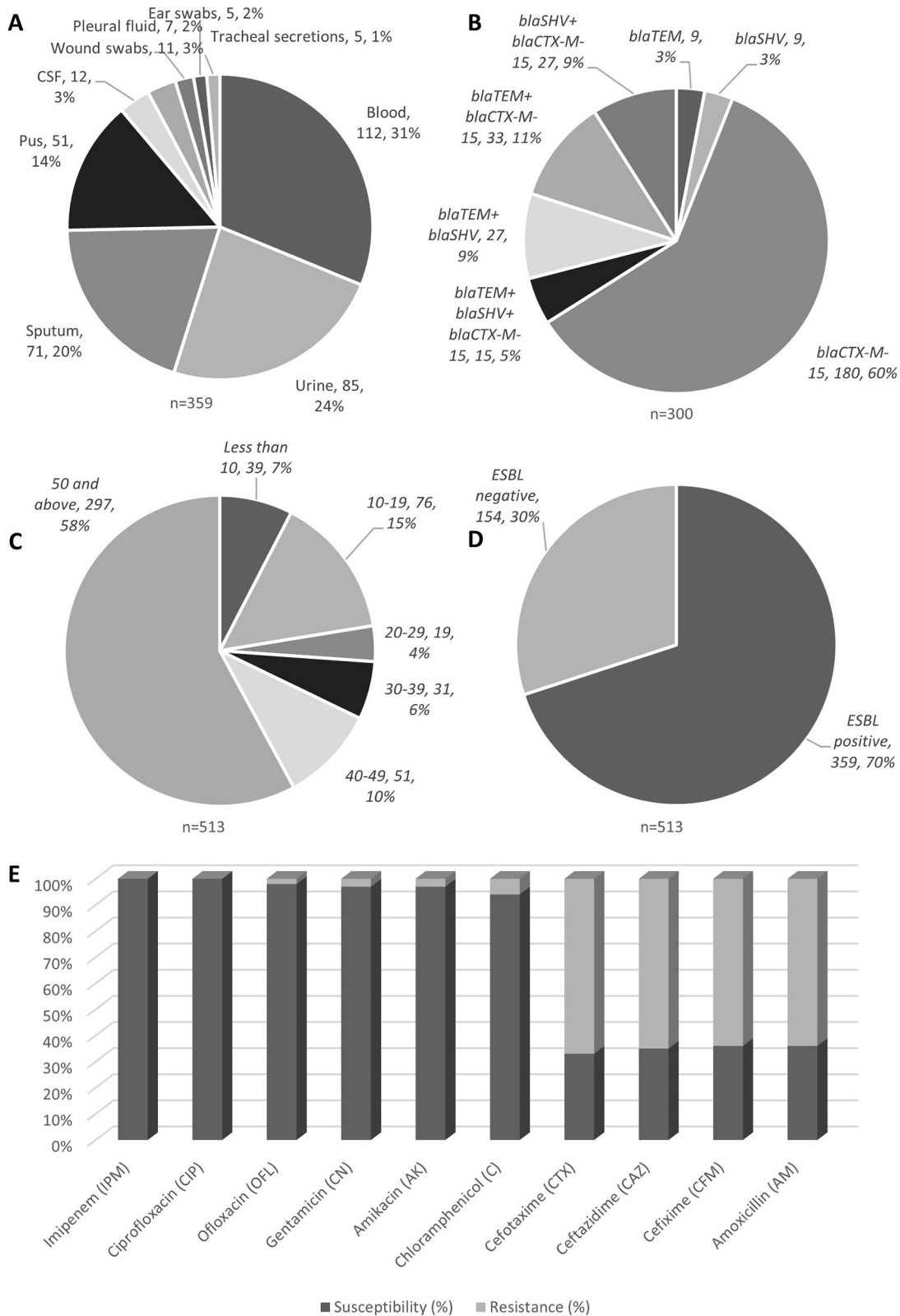
CTX-M-type  $\beta$ -lactamase, has become more predominant than conventional SHV and TEM-type ESBL, which have covered an extensive range of clinically significant bacteria and over a wide environmental area.<sup>6</sup> Moreover, strains that yield ESBL often reveal resistance to antibiotics belonging to other classes (i.e. aminoglycosides, quinolones, and sulfonamides); making its management more complicated.<sup>7</sup>

Consequently, this class of bacteria makes a need to ensure the reporting of ESBL producers in clinical isolates and detection of newly emerging drug resistant isolates. The changing trends of drug resistant ESBL-producing *K. pneumoniae* need time to watch for management, confinement and isolation of patients in hospitals before discharge. It is also necessary to monitor the prevalence and types of ESBLs and define the appropriate therapeutic options accordingly. Hence, the purpose of the current study was to observe the current trends of ESBL producing *K. pneumoniae* drug resistant patterns and to identify the occurrence of ESBL genes in Sindh region of Pakistan.

## Methods

### Sample collection, inclusion and exclusion criteria

A total of 1458 non-duplicate clinical samples were collected between June 2019 and May 2020 from outpatient and inpatient wards of People's University of Medical and Health Sciences, Nawabshah, Pakistan [tertiary care government hospital with 1000 beds] through its diagnostics and research laboratory. Patients referred by their primary physicians for screening tests (such as blood complete count and erythrocyte sedimentation rate (ESR) were recruited in the study. The clinical samples including blood, sputum, urine, cerebrospinal fluid, tracheal secretions, and pus samples were collected by standard techniques (Figure 1A). The clinical samples, which yielded the *K. pneumoniae* were selected for further assessment, while others were excluded. The demographic data was retrieved from clinical survey that included age, gender, specimen type, town, and susceptibility patterns (Table 2).



**Figure 1.** *K. pneumoniae* isoaltes obtained from various clinical samples showed high prevalence as blood borne infections (A); genotypic distribution showed *blaCTX-M-15* to be highly prevalent (B); age group wise analysis showed elderly group of 50 years old and above showed high incidences (C); ESBL positivity to be 70% among all isolates tested (D), with their antibiotic susceptibility profile (E).

### Isolation and identification

The samples were not specifically collected for this research, they were used after the completion of routine laboratory investigations upon the permission of the concerned laboratory's authority. The isolation of bacteria was carried out by conventional bacteriological techniques and identification was confirmed by API 20E identification kit (BioMerieux, France).<sup>8</sup> As many as 513 *K. pneumoniae* isolates from various clinical samples and sites (Figure 1A) were obtained and included in the present study.

### Antimicrobial susceptibility test

Antimicrobial susceptibility testing (AST) was performed by Kirby Bauer Disk diffusion method on Mueller-Hinton agar plates (Beckton Dickinson, Sparks, MD, USA) according to Clinical Laboratory Standards Institute (CLSI).<sup>8</sup> Disks (Beckton Dickinson) comprising of the following antibiotics were used: cefixime 30 µg, amoxicillin 30 µg, ceftazidime 30 µg, cefotaxime 30 µg, Ofloxacin 10 µg, imipenem 10 µg, amikacin 30 µg, gentamicin 10 µg, ciprofloxacin 5 µg and chloramphenicol 30 µg.

### ESBL screening and confirmation by phenotypic methods

Double disk diffusion methods were used to detect ESBL using four antibiotic disks: cefotaxime 30 µg, cefotaxime with clavulanic acid 10 µg, ceftazidime 30 µg, and ceftazidime with clavulanic acid 10 µg. A more than 5 mm increase in zone of inhibition width for whichever antimicrobial agent tested in combination with clavulanic acid against its zone of inhibition when tested alone was labelled as ESBL positive.<sup>9</sup> The inoculum and incubation conditions were the same as recommended for standard disk diffusion method. *K. pneumoniae* ATCC 700603 (American type culture collection, ATCC, Manassas, USA) was used as quality control strain.

### Detection of ESBL genotypes by PCR amplification

Bacterial genomic DNA was separated by boiling the young bacterial colony in 50µl distilled water in a hot bath set at 98°C. This DNA template was used for all PCR reactions. Amplification of *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub> and *bla*<sub>CTX-M-15</sub> ESBL genes was done on GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). The primers used are given in Table 1. The PCR yields were run on 1% agarose gels and observed in UV light. Purified PCR products of CTX-M genes were sequenced on an Applied Biosystems 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA).

**Reaction mixture:** A 30 µl total reaction mixture consisted of the following: 2 µl of DNA template; 1.5 µl of 10 µM forward and reverse primer; 1.5 µl of Sigma brand Red Taq; 20.2 µl of pure water; 3 µl of PCR buffer with MgCl<sub>2</sub>; and 0.3 µl of 10 mM dNTPs.

### Data analysis

The data were analyzed by IBM SPSS Statistics for Windows, Version 21.0, Inc. USA Statistical Package of Social Sciences (SPSS) version 21.0. Continuous and categorical variables were analyzed by *t*-test and Chi-square testing, respectively (these tests can also be carried out on the open source software R). Variance of resistance levels of various antimicrobials in ESBL producing isolates versus non-ESBL producing isolates were calculated by the Fisher exact test. The data was analyzed at 95% Confidence interval ( $p \leq 0.05$  indicates a significant difference).

### Results

During the study period, a total of 513 (35.18%) *K. pneumoniae* were isolated. From those 513 positive cultures, 411 (80.1%) belonged to males and 102 (19.8%) to females ( $p = 0.0001$ ). The demographic characteristics of patients including age, gender, rural or urban, and clinical interventions are summarized in Table 2. The results show that the

**Table 1. List of primers used.**

ESBL Gene	Primer	Sequence (5'-3')*	Product size (bp)	Annealing temperature (°C)
<i>bla</i> <sub>TEM</sub>	Forward	ATGAGTATTCAACATTTCCGTG	861	55
	Reverse	TTACCAATGCTTAATCAGTGAG		
<i>bla</i> <sub>SHV</sub>	Forward	ATTTGTCGCTTCTTTACTCGC	1051	60
	Reverse	TTTATGGCGTTACCTTTGACC		
<i>bla</i> <sub>CTX-M-15</sub>	Forward	CACACGTGGAATTTAGGGACT	996	50
	Reverse	GCCGTCTAAGGCGATÁAACA		

\*Ref: Muzahed *et al.*, 2008 (32).

**Table 2. Demographic characteristics and interventions in study population (n = 513).**

Specimen	<i>Klebsiella pneumoniae</i>	
	N	%
Age (mean $\pm$ SD)	48.5 $\pm$ 8.9 (range: 9-56 years)	
Male	411	80.1
Female	102	19.8
Rural	124	24.17
Urban	389	75.8
Children	115	22.41
Old age	398	77.58
History of interventions		
Nasogastric intubation	76	14.81
Intravenous line	319	62.18
Central venous line	11	2.14
Urinary catheters	217	42.3
Tracheotomy	9	1.75
Endotracheal intubation	23	4.39
Surgery	19	3.7

majority of patients were in the older age group (50 and above) and lived in urban areas [Figure 1B](#). The categories of patient's ages have been shown in [Figure 1C](#). The mean age of the total study subjects was 48.5  $\pm$  8.9 years.

Out of 513 isolates 359 (69.9%) were ESBL producing *K. pneumoniae* ([Figure 1D](#)). The incidences of ESBL was higher in males as compared to females. The frequency of ESBL producing *K. pneumoniae* from different samples (n = 359) has been shown in [Table 3](#) and [Figure 1A](#). The means  $\pm$  SD age of patients with ESBL positive and negative *K. pneumoniae* was 47  $\pm$  17.5 years and 47.5  $\pm$  12.8 years, respectively (p = 0.093).

The results show that 64% of *K. pneumoniae* in our sample were resistant to Amoxicillin and Cefixime. In addition, an important drop of 30–40% was perceived in the susceptibility for all Cephalosporins. However, *K. pneumoniae* presented a dissimilar sensitivity rate to Chloramphenicol, Ofloxacin, Gentamicin and Amikacin with 94%, 98%, 97%, and 97%, respectively and depicted in [Table 4](#).

Regarding the PCR and DNA sequencing of ESBL genotypes, it was found that all of the ESBL-positive *K. pneumoniae* isolates had one or more ESBL genes that were tested in the present study and presented in [Table 5](#). Overall, 83.56% (300/359) of *K. pneumoniae* isolates were positive for one or more ESBL genes. The PCR assay and DNA sequencing

**Table 3. Frequency of ESBL - *K. pneumoniae* in various clinical specimens (n = 513).**

Specimen	ESBL Positive <i>Klebsiella pneumoniae</i>	
	N	%
Blood	112	21.8
Sputum	71	13.8
Pus	51	9.9
Cerebrospinal fluid	12	2.3
Ear swabs	5	0.97
Wound swabs	11	2.14
Pleural fluid	7	1.36
Urine	85	16.5
Tracheal secretions	5	0.97
Total	359/513	69.9

**Table 4. Antibiotic resistance pattern.**

Name of antibiotics	Susceptibility (%)	Resistance (%)
Ceftazidime (CAZ)	35	65
Cefotaxime (CTX)	33	67
Cefixime (CFM)	36	64
Amoxicillin (AM)	36	64
Ofloxacin (OFL)	98	02
Imipenem (IPM)	100	00
Ciprofloxacin (CIP)	100	00
Chloramphenicol (C)	94	06
Gentamicin (CN)	97	03
Amikacin (AK)	97	03

**Table 5. Prevalence of ESBL genotypes.**

Genotypes	Incidences n (%) <sup>*</sup>
<i>bla</i> <sub>TEM</sub>	9 (3%)
<i>bla</i> <sub>SHV</sub>	9 (3%)
<i>bla</i> <sub>CTX-M-15</sub>	180 (60%)
<i>bla</i> <sub>TEM+</sub> <i>bla</i> <sub>SHV+</sub> <i>bla</i> <sub>CTX-M-15</sub>	15 (5%)
<i>bla</i> <sub>TEM+</sub> <i>bla</i> <sub>SHV</sub>	27 (9%)
<i>bla</i> <sub>TEM+</sub> <i>bla</i> <sub>CTX-M-15</sub>	33 (11%)
<i>bla</i> <sub>SHV+</sub> <i>bla</i> <sub>CTX-M-15</sub>	27 (9%)

\*Overall Total CTX-M-15 is 255 [85]; SHV = 78 [26] and TEM = 84 [28].

results indicated the following frequencies of ESBL genotypes: 85% *bla*<sub>CTX-M-15</sub> gene, 26% *bla*<sub>SHV</sub> gene, and 28% *bla*<sub>TEM</sub> gene.

## Discussion

ESBL's are the major causes of  $\beta$ -lactam antibiotic resistance, particularly in *K. pneumoniae*, which is amongst the most common gram-negative bacteria belonging to *Enterobacteriaceae* families.<sup>10</sup> A study from Ziauddin University Karachi, Pakistan has showed that ESBL producing *K. pneumoniae* frequency is 84.16% amongst reported cases.<sup>11</sup> Similarly, Saleem et al. (Aga Khan University, Karachi, Pakistan) have reported a frequency of 80% of ESBL producing *K. pneumoniae*.<sup>12</sup> These findings were higher as compared to the results presented in the current study, which showed that the frequency of ESBL producing *K. pneumoniae* is 69.6% with a higher incidence in male than in female patients. In this respect, the frequency of EBSL producing *K. pneumoniae* varies from one institution/hospital to another. Several factors could govern this variation such as; the use of antibiotics, drug dose, infection control measures, treating physicians, and the duration of drug therapy - all of these elements may cause a difference in results. The frequency of ESBL producing *K. pneumoniae* in previous research conducted at Aga Khan University Karachi, Pakistan was 30.1% and 47.8%, which is low compared to the present study, as well as, previous studies conducted by Mansouri et al.<sup>10</sup> and Ejaz et al.<sup>13</sup> A Pakistani study found a frequency of ESBLs producing *K. pneumoniae* of 70%,<sup>14</sup> which is in agreement with our study.

Carbapenems are often the final influential therapy of choice to treat infections resulting from MDR *Enterobacteriaceae*. Based on our study and other studies, 100% sensitivity was seen with Imipenem, which have been found to be the most effective antibiotics on the isolates that produce ESBLs. This is an important result of the present study because many infections can be treated with Carbapenems.<sup>12,15</sup>

*bla*<sub>CTX-M</sub> is a predominant genotype in this area of the subcontinent. A further study from Pakistan has reported that 72% of ESBL producing strains had the *bla*<sub>CTXM-15</sub> gene, which was higher than the prevalence of *bla*<sub>CTX-M</sub> gene

reported in the present study.<sup>16</sup> Limited studies from different regions have also shown a higher prevalence of the *bla*CTX-M genotype amongst strains including 84.7% (Chile), whereas a very high prevalence of 98.8% was reported in China and, significantly, a low prevalence of 13.6% in Tanzania.<sup>17–19</sup>

In conclusion, the findings of the current study emphasizes the urgent need for screening and surveillance of ESBL producing *K. pneumoniae* in routine microbiology laboratories for early detection, prompt intervention, and successful clinical management before exacerbation of the infection magnitude. In this regard, the current findings may be a valuable contribution to the medical literature providing physicians with an updated prevalence status of ESBL producing *K. pneumoniae*. Larger studies need to be done in various geographical regions of the country to better define the molecular epidemiology of ESBL-producing *K. pneumoniae* and its clinical implications.<sup>20</sup> Finally, the limited sample size along with the limited time frame are two common limitations, which could affect the outcomes of the study.

## Acknowledgement

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## Ethical approval

Prior to the study we contacted the institutional review board representative (PUMHS, IRB) for discussion. The IRB representative confirmed that since patients are not directly involved and the samples are not collected specifically for this research there is no need for IRB approval. Patient data consent was not required as we didn't retrieve any personal information, such as medical record numbers, national ID, and names, from patients. The data was completely anonymous and thus does not violate the privacy or confidentiality of any of the patients.

## Data availability

All data underlying the results are available as part of the article and no additional source data are required.

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# Open Peer Review

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**Mubashir Zafar**

Aga Khan University, Karachi, Pakistan

All comments are addressed. I recommend article for indexing.

**Competing Interests:** No competing interests were disclosed.

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Reviewer Report 16 November 2021

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**Abbas Farahani** 

Infectious and Tropical Diseases Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

All comments and corrections have been considered by the authors.

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Microbiology

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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**Version 2**

Reviewer Report 27 August 2021

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**Mubashir Zafar**

Aga Khan University, Karachi, Pakistan

The article is good but it needs major revision.

First, the title of the article should be revised as Epidemiological Surveillance of CTX-M-15-producing *Klebsiella pneumoniae* among patients in teaching hospital (add city and country name).

**Major revision****Abstract**

- The objective is different from the title of the study.
- Don't use the abbreviation in the abstract.
- The method in the abstract is incomplete.
- The result and conclusion are also incomplete information in the abstract.

**In the introduction section**

- Relevant studies are absent, the rationale of the study is very weak

**In method section**

- Study setting, sampling technique, sample size were absent.
- The data collection procedure is incomplete and not standard.
- Data analysis should be revised.

**In result section**

- A demographic table is not needed and should be deleted.

**The discussion section is incomplete**

- Limitation of study is absent
- Conclusion of study should be revised
- Reference should be the latest

**Minor revision**

- The article would benefit from being revised for formatting and use of language.

**Is the work clearly and accurately presented and does it cite the current literature?**

Partly

**Is the study design appropriate and is the work technically sound?**

No

**Are sufficient details of methods and analysis provided to allow replication by others?**

No

**If applicable, is the statistical analysis and its interpretation appropriate?**

No

**Are all the source data underlying the results available to ensure full reproducibility?**

Partly

**Are the conclusions drawn adequately supported by the results?**

No

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Preventive Medicine specialist

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.**

Reviewer Report 02 August 2021

<https://doi.org/10.5256/f1000research.58553.r90226>

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**SUPRIT DESHPANDE** 

HIV Vaccine Translational Research Laboratory, NCR Biotech Science Cluster, Translational Health Science & Technology Institute, Faridabad, Haryana, India

There is no mention about ESBL positive *K. pneumoniae* in the Figure 1A description/legend, whereas the same has been described in the running text.  
unaddressed

Clinical relevance of demographic characteristics with the results obtained in this study is not described clearly by the authors in discussion section. This needs to be elaborated."  
unaddressed

As per the study it's not only Carbapenems but also quinolones (Ciprofloxacin) also that showed their effectiveness with 100% sensitivity. Authors has missed to point it out and describe about it in the running text.

unaddressed

Relevant references needs to be quoted for conventional bacteriological techniques in methods  
unaddressed

As per the Fig 1E, the authors have tested different classes of antibiotics, here what was the rationale behind choosing these specific antibiotics? The results illustrates that the more percent resistance was mainly observed in the antibiotics belongs to class of cephalosporins, is there any clinical relevance for this kind of observation, this needs to be discussed in the discussion section with relevant references if available.

unaddressed

Authors mentioned in the method that the genomic DNA was separated upon boiling the bacterial colony at 98 degrees Celsius and the same DNA was used for PCR amplification of ESBL genes. The ESBL genes are mainly encoded by plasmid DNA. Here, in this study the authors used which DNA (bacterial genomic DNA or plasmid DNA) for PCR amplification to understand the genetic diversity of ESBL genes

Needs to be addressed

The highest percent of ESBL was found in blood specimens, is there any scientific reason for this? If yes, it needs to be described in the discussion part.

Unaddressed

Authors mentioned in the method that the genomic DNA was separated upon boiling the bacterial colony at 98 degrees Celsius and the same DNA was used for PCR amplification of ESBL genes. The ESBL genes are mainly encoded by plasmid DNA. Here, in this study the authors used which DNA (bacterial genomic DNA or plasmid DNA) for PCR amplification to understand the genetic diversity of ESBL genes?

Unaddressed

**Is the work clearly and accurately presented and does it cite the current literature?**

No

**Is the study design appropriate and is the work technically sound?**

No

**Are sufficient details of methods and analysis provided to allow replication by others?**

No

**If applicable, is the statistical analysis and its interpretation appropriate?**

No

**Are all the source data underlying the results available to ensure full reproducibility?**

No

**Are the conclusions drawn adequately supported by the results?**

No

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** HIV molecular virology , immunology and antibody discovery

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Reviewer Report 28 July 2021

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**Abbas Farahani** 

Infectious and Tropical Diseases Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

The revised manuscript has been improved, however, before it can be accepted for publication in the journal, I require of authors to revise it before indexing. Comments to authors:

- Please provide the accession numbers for ESBL genotypes.
- In general, the discussion can be structured better. There is a substantial overlap and repetition between the Result and the Discussion sections.

**Is the work clearly and accurately presented and does it cite the current literature?**

No

**Is the study design appropriate and is the work technically sound?**

No

**Are sufficient details of methods and analysis provided to allow replication by others?**

No

**If applicable, is the statistical analysis and its interpretation appropriate?**

No

**Are all the source data underlying the results available to ensure full reproducibility?**

No

**Are the conclusions drawn adequately supported by the results?**

No

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Microbiology

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

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### Version 1

Reviewer Report 05 July 2021

<https://doi.org/10.5256/f1000research.56580.r88330>

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**Abbas Farahani** 

Infectious and Tropical Diseases Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

#### Summary:

This research article seeks to isolate and characterize ESBL-producing *K. pneumoniae* from inpatients and outpatients. The ESBL genotype and molecular relatedness of strains identified here were done using standard and acceptable methods and procedures. *K. pneumoniae* isolates were identified in 35.18% (n=513) of all samples. More than half (69.9%) of all isolates were ESBL producers and all of the ESBL-positive *K. pneumoniae* isolates had one or more ESBL genes. Their study showed that CTX-M-15 was the major prevalent ESBL type among the isolates.

#### Comments:

- Teaching hospital needs description of the hospital, number of beds, number of admission and other information about the hospital is required.
- The authors claimed that to have used the CLSI guideline, while reference number 8 is related to the European Committee on Antimicrobial Susceptibility Testing (EUCAST)?
- Where did you source your antibiotics from?
- The concentration of each reaction in PCR must be added.
- Please provide information about quantifying and qualify DNA samples.

- Please add company and country for SPSS software.
- The sequenced products of the isolates need to be deposited in a database (i.e. Genbank) and accession numbers need to be given.
- Provide the accession numbers of the sequenced products in the article.
- In Table 2; mean  $\pm$  SD is  $47 \pm 17.5$  while in text is  $48.5 \pm 8.9$  years? Please explain.
- In the title of the article the authors have mentioned that isolates collected from inpatients and outpatients of a teaching hospital, but in the main text is no mention of them and the number of isolates in each group is not mentioned? Please explain.
- Finally, I recommend merging both Results and Discussion sections for better clarity and impact, and move to Rapid Communication submission.

**Is the work clearly and accurately presented and does it cite the current literature?**

No

**Is the study design appropriate and is the work technically sound?**

No

**Are sufficient details of methods and analysis provided to allow replication by others?**

No

**If applicable, is the statistical analysis and its interpretation appropriate?**

No

**Are all the source data underlying the results available to ensure full reproducibility?**

No

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Microbiology

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.**

Author Response 08 Jul 2021

**Saeed Sattar Shaikh**, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia



We thank the reviewer for taking time and reviewing our article. We have revised our manuscript to address the comments. Please, find below our responses to reviewer comments.

- *Teaching hospital needs description of the hospital, number of beds, number of admission and other information about the hospital is required.*
- **Author's Response:** Details of hospital updated.
  
- *The authors claimed that to have used the CLSI guideline, while reference number 8 is related to the European Committee on Antimicrobial Susceptibility Testing (EUCAST)?*
- **Author's Response:** Replaced with new reference.
  
- *Where did you source your antibiotics from?*
- **Author's Response:** Updated the details of antibiotics.
  
- *The concentration of each reaction in PCR must be added.*
- **Author's Response:** PCR reaction mixture details are added.
  
- *Please provide information about quantifying and qualify DNA samples.*
- **Author's Response:** The information and data were not mentioned because, for PCR reaction purified DNA was not required and crude DNA was sufficient for detection.
  
- *Please add company and country for SPSS software.*
- **Author's Response:** Updated the details of SPSS software.
  
- *The sequenced products of the isolates need to be deposited in a database (i.e. Genbank) and accession numbers need to be given.*
- **Author's Response:** As per our Institutional policy, we have to deposit all research data to our institution. Hence it takes much administrative procedures before the institution deposits it to GenBank.
  
- *Provide the accession numbers of the sequenced products in the article.*
- **Author's Response:** As per our Institutional policy, we have to deposit all research data to our institution. Hence it takes much administrative procedures before the institution deposits it to GenBank.
  
- *In Table 2; mean  $\pm$  SD is  $47 \pm 17.5$  while in text is  $48.5 \pm 8.9$  years? Please explain.*
- **Author's Response:** The correct one is  $48.5 \pm 8.9$  years; and is now corrected.
  
- *In the title of the article the authors have mentioned that isolates collected from inpatients and outpatients of a teaching hospital, but in the main text is no mention of them and the number of isolates in each group is not mentioned? Please explain.*
- **Author's Response:** Authors trying to indicate; Inpatient and outpatients as all patients attending the hospitals. We have updated the title of the article to avoid confusion.

- *Finally, I recommend merging both Results and Discussion sections for better clarity and impact and move to Rapid Communication submission.*
- **Author's Response:** We do not have any problem in merging results and discussion if journal format allows us.

**Competing Interests:** None to declare

Reviewer Report 05 July 2021

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**SUPRIT DESHPANDE**

HIV Vaccine Translational Research Laboratory, NCR Biotech Science Cluster, Translational Health Science & Technology Institute, Faridabad, Haryana, India

In this manuscript "Characterization of CTX-M-15-Klebsiella pneumoniae from inpatients and outpatients of a teaching hospital" by Muzahed *et al.*, the authors studied and surveyed the prevalence of ESBL producing genes in *Klebsiella pneumoniae* infections. The authors have screened *K. pneumoniae* from 1458 clinical samples from the individuals enrolled under a different study. Increase in number of cases due to bacterial drug resistance has become a severe cause of global concern. Here, authors have screened the *K. pneumoniae* isolates and with the help of genotyping they tried to identify the distribution of different class of ESBL genes and their prevalence.

1. This paper imparts more of a kind "molecular epidemiological surveillance" of ESBL producing genes in one of the predominant nosocomial pathogen *K. pneumoniae*, whereas the title of the paper is about characterization of one of the ESBL producing genes (CTX-M). Author needs to re-think about the title of this paper as the existing title seems not suitable for the kind of study actually done and reported through this manuscript
2. Since the samples were not specifically collected for this study, then the inclusion and exclusion criteria would have been not as per this current study? This needs to be clarified. Also, it would be better to shift the entire sentence about sample collection from isolation and identification paragraph to the paragraph on participants inclusion and exclusion criteria.
3. Under methods, the second paragraph on sample collection, inclusion and exclusion criteria needs to be shifted first and the present first paragraph on isolation and identification needs to be shifted second.
4. Double parentheses for ESR in inclusion and exclusion criteria paragraph in methods

section need to be removed.

5. Author mentioned Kirby bar method, it needs to be mentioned as Kirby Bar Disk diffusion method.
6. Under methods section in the paragraph on ESBL screening and confirmation by phenotypic methods, in the last but one line the standard disc diffusion where "method" is missing needs to be written.
7. There is no mention about ESBL positive *K. pneumoniae* in the Figure 1A description/legend, whereas the same has been described in the running text.
8. Figure 1B is not described in the running text. Also in figure there is confusion with the numbering corresponds to the genes, for instance in TEM, SHV and CTX-M there should be three numbers (one number for each gene) but only two numbers are given along with the percentage. Please check and confirm.
9. Clinical relevance of demographic characteristics with the results obtained in this study is not described clearly by the authors in discussion section. This needs to be elaborated.
10. As per the study it's not only Carbapenems but also quinolones (Ciprofloxacin) also that showed their effectiveness with 100% sensitivity. Authors has missed to point it out and describe about it in the running text.
11. The highest percent of ESBL was found in blood specimens, is there any scientific reason for this? If yes, it needs to be described in the discussion part.
12. What is the denominator for 411 males and 102 females clinical specimens from whom *K. pneumoniae* were isolated? If the sample sizes for male and female donors were different then before coming to the conclusion saying that in males it was higher than in females it needs to be proved statistically. Authors needs to clarify this.
13. As per the Fig 1E, the authors have tested different classes of antibiotics, here what was the rationale behind choosing these specific antibiotics? The results illustrates that the more percent resistance was mainly observed in the antibiotics belongs to class of cephalosporins, is there any clinical relevance for this kind of observation, this needs to be discussed in the discussion section with relevant references if available.
14. Authors mentioned in the method that the genomic DNA was separated upon boiling the bacterial colony at 98 degrees Celsius and the same DNA was used for PCR amplification of ESBL genes. The ESBL genes are mainly encoded by plasmid DNA. Here, in this study the authors used which DNA (bacterial genomic DNA or plasmid DNA) for PCR amplification to understand the genetic diversity of ESBL genes?
15. Authors mentioned the amplicons were sequenced to identify the genetic diversity of different ESBL producing genes, I am not sure about the journal's policy and whether these need to be submitted to the GenBank?

16. Relevant references needs to be quoted for conventional bacteriological techniques in methods.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**

I cannot comment. A qualified statistician is required.

**Are all the source data underlying the results available to ensure full reproducibility?**

Partly

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** HIV molecular virology , immunology and antibody discovery

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Author Response 08 Jul 2021

**Saeed Sattar Shaikh**, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

We thank you for your valuable comments and suggestions. We have revised our manuscript accordingly. Please, find below our responses to reviewer comments.

- *This paper imparts more of a kind "molecular epidemiological surveillance" of ESBL producing genes in one of the predominant nosocomial pathogen K. pneumoniae, whereas the title of the paper is about characterization of one of the ESBL producing genes (CTX-M). Author needs to re-think about the title of this paper as the existing title seems not suitable for the kind of study actually done and reported through this manuscript*
- **Author's Response:** Thank you for your suggestions we have modified title as suggested.
- *Since the samples were not specifically collected for this study, then the inclusion and exclusion criteria would have been not as per this current study? This needs to be clarified. Also, it would be better to shift the entire sentence about sample collection from isolation and identification paragraph to the paragraph on participants inclusion and exclusion*

*criteria.*

- **Author's Response:** The inclusion and exclusion criteria were set based on our focused organism that is *Klebsiella pneumoniae*. Also, we have shifted the entire sentence about sample collection from isolation and identification paragraph to the paragraph on participants inclusion and exclusion criteria.
- *Under methods, the second paragraph on sample collection, inclusion and exclusion criteria needs to be shifted first and the present first paragraph on isolation and identification needs to be shifted second.*
- **Author's Response:** Updated shifted as suggested.
- *Double parentheses for ESR in inclusion and exclusion criteria paragraph in methods section need to be removed.*
- **Author's Response:** Thank you, removed.
- *Author mentioned Kirby bar method, it needs to be mentioned as Kirby Bar Disk diffusion method.*
- **Author's Response:** Thank you, updated as suggested.
- *Under methods section in the paragraph on ESBL screening and confirmation by phenotypic methods, in the last but one line the standard disc diffusion where "method" is missing needs to be written.*
- **Author's Response:** Thank you, updated as suggested.
- *There is no mention about ESBL positive K. pneumoniae in the Figure 1A description/legend, whereas the same has been described in the running text.*
- **Author's Response:** Thank you, updated figure legend as suggested.
- *Figure 1B is not described in the running text. Also in figure there is confusion with the numbering corresponds to the genes, for instance in TEM, SHV and CTX-M there should be three numbers (one number for each gene) but only two numbers are given along with the percentage. Please check and confirm.*
- **Author's Response:** figure 1B is now mentioned in running text. The names of the genes have been written as per the standard procedures followed in previous literatures.
- *Clinical relevance of demographic characteristics with the results obtained in this study is not described clearly by the authors in discussion section. This needs to be elaborated.*
- **Author's Response:** The aim and objectives of the study were to perform molecular epidemiology of the isolates regardless of their clinical sources and clinical presentations. This was beyond the scope of clinical correlations. However, existing data were being used for further study and analysis where additional publication is being tried.
- *As per the study it's not only Carbapenems but also quinolones (Ciprofloxacin) also that showed their effectiveness with 100% sensitivity. Authors has missed to point it out and describe about it in the running text.*

- **Author's Response:** Carbapenems are often the final influential therapy of choice to treat infections resulting from MDR Enterobacteriaceae that is why the authors have only concentrated on it.
- *The highest percent of ESBL was found in blood specimens, is there any scientific reason for this? If yes, it needs to be described in the discussion part.*
- **Author's Response:** Because the clinical samples were mostly from blood.
- *What is the denominator for 411 males and 102 females clinical specimens from whom K. pneumoniae were isolated? If the sample sizes for male and female donors were different then before coming to the conclusion saying that in males it was higher than in females it needs to be proved statistically. Authors needs to clarify this.*
- **Author's Response:** The dominator is 513, which were positive K. pneumoniae Out of which 411 were males and 102 females.
- *As per the Fig 1E, the authors have tested different classes of antibiotics, here what was the rationale behind choosing these specific antibiotics? The results illustrates that the more percent resistance was mainly observed in the antibiotics belongs to class of cephalosporins, is there any clinical relevance for this kind of observation, this needs to be discussed in the discussion section with relevant references if available.*
- **Author's Response:** The antibiotics were selected according to CLSI guidelines, Physician's desk reference, Medical Letters and Peer-reviewed literature.
- *Authors mentioned in the method that the genomic DNA was separated upon boiling the bacterial colony at 98 degrees Celsius and the same DNA was used for PCR amplification of ESBL genes. The ESBL genes are mainly encoded by plasmid DNA. Here, in this study the authors used which DNA (bacterial genomic DNA or plasmid DNA) for PCR amplification to understand the genetic diversity of ESBL genes?*
- **Author's Response:** Bacterial genomic DNA was used for PCR amplification. Whereas Trans-conjugation experiments were performed to identify conjugal resistance transfer. Which will be reported in next possible publication.
- *Authors mentioned the amplicons were sequenced to identify the genetic diversity of different ESBL producing genes, I am not sure about the journal's policy and whether these need to be submitted to the GenBank?*
- **Author's Response:** As per our Institutional policy, we have to deposit all research data to our institution. Hence it takes much administrative procedures before the institution deposits it to GenBank.
- *Relevant references needs to be quoted for conventional bacteriological techniques in methods.*
- **Author's Response:** Thank you, reference added as suggested.

**Competing Interests:** None to Declare

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