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Study of plasmid-mediated Amp-C beta lactamases activity among klebsiella pneumoniae isolated from patients of Tanta university hospitals

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Abstract
Background: The primary enzymatic mechanism for antibiotic resistance in Klebsiella pneumoniae (KP) is Amp-C beta lactamases.
Aim: Finding the Amp-C-Beta-Lactamase gene that causes antibiotic resistance in Klebsiella pneumonia isolated from patients at Tanta University hospitals was the goal of the current study.
Methods and subjects: B-lactamase at 100 Amp-C The synthesis of Amp-C was examined in Klebsiella pneumoniae. The majority of Amp-C positive strains were successfully detected by three phenotypic Amp-C confirmation assays (Amp-C E test, the disc approximation test, and Amp C EDTA disc). Multiplex PCR was then used to molecularly detect plasmid-mediated Amp-C in order to detect the CMY-1, CMY-2, and FOX-1 genes.
Results: Among 100 MDR *Klebsiella pneumoniae* isolates, positive isolates for Amp-C by phenotypic tests were detected in 34 isolates (34%). Then by application of multiplex PCR on the positive isolates for phenotypic tests there were (26) isolates out of (34) isolates harbored Amp-C genes including CMY-1which was detected in (65.3%) (17/26), followed by CMY-2 was detected in (50.0%) (13/26), finally FOX-1 genes were detected in (34.6%) (9/26), so CMY-1 gene was the predominant type in all isolates.
Conclusion: It was determined that: The Tanta University Hospital has a growing number of isolates of Klebsiella pneumoniae strains that produce Amp-C. Therefore, a trustworthy epidemiological study of their spread in hospitals would need molecular identification of the genes expressing Amp-C.

Keywords: *Klebsiella pneumoniae*, Amp-C βeta-lactamases, PCR

Introduction

Antibiotic resistance is increasing at a frightening speed and is really a matter of serious concern. Antibiotic resistance presents a significant therapeutic challenge, both in healthcare facilities and our local communities, due to widespread bacterial resistance to numerous medications.

Drug resistance mechanisms in Gram-negative bacteria include the production of ESBLs, Amp-C lactamases, efflux mechanisms, and porin deficiencies. Enzymes such as Amp-C lactamases and ESBLs are often found in clinical laboratory settings and contribute to resistance.

Amp-C lactamases hold significant clinical relevance due to their unique properties, which include the ability to render cephamycins and other extended-spectrum cephalosporins ineffective, while also displaying resistance to clavulanic acid but susceptibility to cefepime and carbapenems, and an insensitivity to β-lactamase inhibitors [2].

Detecting Amp-C is essential for improving patient care and obtaining reliable epidemiological information. Inconsistent test findings, particularly in phenotypic assays, derive from the lack of set criteria by clinical and laboratory standard organisations for diagnosing Amp-C mediated resistance in gram-negative bacterial isolates [3]. Multiplex PCR is widely regarded as the most reliable method for identifying plasmid-mediated Amp-C β-lactamases [4].

Encoded genes for Amp-C can be found on either plasmids or the bacterial chromosome, and transmissible plasmids that carry Amp-C genes can occur in bacteria that lack or do not

adequately express the chromosomal Amp-C gene, such as Escherichia coli, Klebsiella spp., and Proteus mirabilis. The presence of these genes on plasmids facilitated their spread among members of the Enterobacteriaceae family [5].

E. coli and Klebsiella species, which generate Amp-C through plasmids, are often associated with multidrug resistance, thereby limiting treatment options. The initial documented instance of plasmid-mediated Amp-C β-lactamases in K. pneumoniae isolates was observed in Seoul, South Korea in 1989. To date, reports of these occurrences have been documented globally and a total of 29 distinct plasmid-mediated Amp-C genes have been identified and deposited in GeneBank [6].

Klebsiella pneumoniae, part of the Enterobacteriaceae group, is regarded as one of the most concerning pathogens associated with antibiotic resistance, and it has been classified as an ESKAPE organism, along with other highly significant multi-drug resistant pathogens [6].

Rapid identification of bacteria that produce beta-lactamase is crucial for two main reasons: it helps to lower patient mortality rates and prevents the spread of these strains within the hospital setting [3].

Aim

The aim of the present work is detection of Genotypic characterization of Amp-C-Beta-Lactamase responsible for antimicrobial resistance among *klebsiella pneumonia* isolated from patients of Tanta University hospitals.

Patients and Methods

The research was conducted over the period from January (2018) to January (2019) within the Clinical Pathology Department of the Faculty of Medicine at Tanta University. The study received approval from the Ethical Committee of Tanta University Hospitals, located in Tanta, Egypt.

Samples from 100 patients were collected, consisting of sputum, endotracheal aspirate, pus from open wounds or abscesses, urine samples from non-catheterized and catheterized patients, blood samples, and bronchoalveolar lavage. These specimens were obtained from Tanta University Hospital patients, collected under strict aseptic conditions in accordance with standard protocols, and processed immediately**.**

All isolates were grown on MacConkey's agar, blood agar, and Cysteine Lactose Electrolytes Deficient (CLED) agar. After that, they were kept in an aerobically-controlled environment at 37 degrees Celsius for 24 hours. Colonies isolated in isolation were assessed microbiologically and characterised using common microbiological methods including colonial morphology, Gram staining, and biochemical tests like triple sugar, Indole, Oxidase, Catalase, Urease, and citrate, as outlined in Cheesebrough [7].

Determining antibiotic resistance profiles

According to the Clinical and Laboratory Standards Institute's 2019 recommendations, a modified Kirby-Bauer disc diffusion test on Muller-Hinton agar plates was used to assess the isolates' sensitivity to antimicrobial drugs.

Identification of Ampicillin-resistant b-lactamase enzymes through phenotypic analysis

1. As directed by the manufacturer, the Amp-C E test for cefotetan susceptibility was conducted. A strip having cefotetan at one end and cefotetan-cloxacillin at the other is used for the Amp-C E test. P8 produces Amp-C beta-lactamase, as indicated by the MIC ratios for cefotetan and cefotetan-cloxacillin. Cefotetan and cefotetan-cloxacillin MIC ratios for P8 are thought to be a good indicator of ampicillin-carbonic b-lactamase synthesis. Cefotetan and cefotetan-cloxacillin MIC ratios of P8 are regarded as positive for the generation of Amp-C b-lactamase.
2. To identify Amp-C production, the disc approximation test was created. E. coli ATCC 25922, a sensitive strain, is used in a standard disc diffusion susceptibility test. An MHA plate's surface is seeded with an inoculum, which is a 0.5 McFarland dilution of bacteria from an overnight blood agar plate. In the middle of the plate was a 30 microgramme ceftazidime disc. Twenty millimetres from the ceftazidime disc were discs containing 10 microgramme imipenem, 30 microgramme cefoxitin, and 20/10 microgramme amoxicillin/clavulanate. After being inverted, the plate was incubated over the whole night at 37°C. A favourable sign of AmpC production will be identified if, during an overnight incubation period, blunting or flattening appears in the zone of inhibition between the ceftazidime disc and the inducing substrates (imipenem, cefoxitin, and amoxicillin/clavulanate disc). Any discernible blunting or flattening of the zone of inhibition between the ceftazidime disc and the inducing substrates, which include imipenem, cefoxitin, and the amoxicillin/clavulanate disc, after an overnight incubation period is indicative of a positive result for AmpC production (8). Any noticeable flattening or blunting of the zone of inhibition between the ceftazidime disc and the inducing substrates (imipenem, cefoxitin, and amoxicillin/clavulanate disc) during an overnight incubation period would be regarded as a favourable result for the synthesis of Amp-C (8).
3. Diffusion test using an EDTA disc for ampicillin (Amp-C). In the test, a bacterial cell is permeabilized with Tris-EDTA, which causes beta-lactamases to be released outside the cell. Amp-C discs were created by applying 20 microlitres of a 1:1 saline and Tris-EDTA mixture on sterile filter paper discs, followed by the discs being allowed to impregnate with Tris-EDTA. In order to permeabilize the bacterial cell and release beta-lactamases into the surrounding environment, the assay uses Tris-EDTA. In order to manufacture Amp-C discs, which are filter paper discs treated with Tris-EDTA, 20 microlitres of a 1:1 saline and Tris-EDTA mixture were applied to sterile filter paper discs. The discs were then allowed to dry before being stored at a temperature between 2 and 8 degrees Celsius. A Mueller-Hinton agar plate's surface. A lawn of cefoxitin-susceptible E. Coli strain ATCC 25922 was used to treat the bacteria using the conventional disc diffusion approach, as detailed in reference (9). Amp-C discs were reconstituted with 20 microlitres of saline solution before to testing, and then several bacterial colonies were applied on the disc. At the inoculation location, a 30-microgram cefoxitin disc was positioned on the Mueller-Hinton agar surface. Next, the infected Amp-C disc was positioned close to the antibiotic disc such that the infected surface touched the agar surface directly. After that, the plate was turned over and allowed to incubate in the ambient air at 35 °C for the whole night. After that, the plate was inverted and allowed to

incubate in ambient air at 35 °C for the whole night. Following incubation, the plates were inspected for indications of either a flattening or a depression of the zone of inhibition, which would result in a positive result if cefoxitin was inactivated, or the absence of distortion, which would indicate that cefoxitin was not considerably inactivated.

Detection of beta-lactamase resistance genes associated with Amp-C.

The genes CMY-1, CMY-2, and FOX-1, which are responsible for Amp-C beta-lactamase activity, were identified by Multiplex PCR in the (34) phenotypically positive isolates as follows:

Molecular detection of Amp-C b-lactamase

A single colony of each organism was put to five milliliters of broth from a blood agar plate, and the mixture was shaken and cultured for twenty hours at 37 °C in order to create the template DNA. After five minutes of centrifugation, a 1.5 ml sample from the overnight culture was recovered. After the supernatant was decanted, the particle was re-suspended in 500 microlitres of distilled water. The organism was heated to 95 °C for 10 minutes to achieve cellular lysis, and the cellular debris was then extracted using centrifugation for 5 minutes. Two microlitres (1/250 of the sample's total volume) of the supernatant served as the amplification template.

Protocol for multiplex

The polymerase chain reaction was carried out in a DNA thermal cycler with 50 individual reactions, with each reaction occurring in a 0.5-ml thin-walled tube. The primers applied to identify the CMY-1 gene were a forward primer with the nucleotide sequence 5-GCT GCT CAA GGA GCA CAG GAT-3, associated with nucleotides 358-378, and a reverse primer with the sequence CAC ATT GAC ATA GGT GTG GTG-3, corresponding to nucleotides 856-877, yielding an anticipated amplicon size of 520 bp. For CMY-2 an expected amplicon size of 462 base pairs was obtained by using the forward primer sequence 5'-TGG CCA GAA CTG ACA GGC AAA-3', which spans nucleotides 478-498, and the reverse primer sequence 5'-TTT TC CTG AAC GTG GCT GGC-3', which corresponds to nucleotides 919-939. For FOX-1 an amplicon of the anticipated length of 190 base pairs was produced using the forward primer 5-AAC ATG GGG TAT CAG GGA GAT G-3, which is situated at nucleotides 1475-11496, and the reverse primer 5-CAA AGC GCG TAA CCG GAT TGG-3, which is placed at nucleotides 1664-1644 [9].

Twenty millimolar Tris-HCl (pH 8.4), fifty millimolar KCl, 0.2 millimolar of each of the deoxynucleoside triphosphates, 1.5 millimolar MgCl2, 0.6 micromolar primers, and 1.25 units of Taq were used in each reaction. 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 0.2 mM of each deoxynucleoside triphosphate, 1.5 mM MgCl2, 0.6 lM primers, and 1.25 U of Taq were included in each reaction.

Life Technologies, a business based in Rockville, Maryland, provides DNA polymerase. Before being covered with mineral oil, 48 microlitres of a master mixture were combined with two microlitres of a DNA template. A three-minute initial denaturation stage at 94 °C preceded the remaining steps in the PCR process.

The procedure comprises 25 cycles, which include primer annealing at 64 degrees Celsius for 30 seconds, primer extension at 72 degrees Celsius for 1 minute, and DNA denaturation at 94 degrees Celsius for 30 seconds. A 7-minute extension step at 72 °C was introduced at the end of the previous cycle. PCR product aliquots, each five microlitres in volume.

Following gel electrophoresis on 2% agarose gels, the samples were stained with 10 microgrammes of ethidium bromide per millilitre and examined under UV transillumination. Life Technologies provided the 100-bp DNA marker that was utilised. Standard PCR mixes were modified to produce controls with no template DNA by replacing water. In a multiplex PCR setup, ideally all primer pairs should have equivalent amplification efficiencies for their specific targets. The desired outcome can be achieved by utilising primers with highly comparable optimal annealing temperatures, namely those with lengths of 18-30 base pairs or more and a GC content of 35-60%, which can be effective and lack notable similarities internally or between one another.

Statistical analysis

Statistical analysis was performed using SPSS v26, which is a product of IBM Inc., located in Chicago, IL, USA. Results were presented for the quantitative variables, including their mean and standard deviation (SD), Qualitative factors were represented using frequencies and percentages (%).

Results

there were (840) isolates showed growth of gram-negative organisms out of them (40%) (336) isolates were *Klebsilla pneumoniae* proved by culture, biochemical reaction. then by application of disc diffusion method there were (30%) (100) isolates multi-drug resistance (MDR), these isolates are collected from different sites of the patients of Tanta university Hospital patients of different age and sex are included in this study, The results of this study were collected, analyzed and tabulated as follow to help us to understand the importance of this organism and its ability for drug resistance which threat our world.

Table 1: Age and sex distribution among the studied patients

	Range	**Mean ± SD**
Age	20 - 80	52.95 ± 8.58
Sex	N	%
Male	63	63
Female	37	37
Total	100	100

Then these isolates were categorized according to the department from which the samples were collected, the importance of this categorization was to know the rate of spread of the *Klebsiella Pneumonia* among these departments and incidence of the patient affection by the organism, It was found that the highest percentage in the Chest department followed by emergency department, Anesthesia department, Neuropsychiatry department, and Internal medicine department.

Table 2: Distribution of isolates among different departments of Tanta University Hospital

Department	N	%
Anesthesia I.C.U	21	21
Chest I.C.U	30	30
Emergency I.C.U	24	24
Internal medicine I.C.U	7	7
Neuropsychiatry I.C.U	18	18
Total	100	100

We also cared about the classification of the origin of the sample to know the most common site in which *Klebsiella pneumoniae* are commonly present and to know the methods of transmission between the patients and transmission of the organism in general, Isolates from sputum and pus showed the largest percentage followed by urine &blood then tracheal tube & bronchoalveolar lavage showed the least percentage.

Table 3: Origin of isolates which gave rise to Klebsiella Pneumoniae

Origin of samples	N	%
Sputum	34	34
Pus & Wound	28	28
Urine	17	17
Blood	14	14
Tracheal tube	4	4
Bronchoalveolar lavage	3	3
Total	100	100

Then the antibiotic discs were applied on the collected 100 isolates after their culture on different media all of them were multi-drug resistance and showed sensitivity to some antibiotics especially Colistin (86%), Tigecycline (80%), Imipenem (60%), Meropenem (46.6%), There were (15) isolates which showed sensitivity to different antibiotics in different ratios Colistin (86%), Tigecycline (80%), Imipenem (60%), Meropenem (46.6%).

The isolates were then subjected to phenotypic assays to see if they produced Amp-C β-lactamases in order to confirm their resistance; the positive samples were 34 percent (34 out of 100), whereas the negative samples were 66 percent (66).

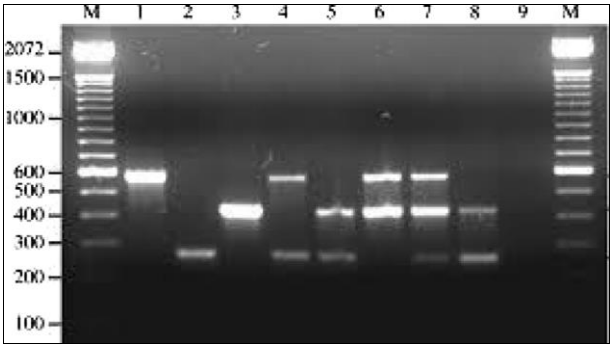
Table 4: indicates the percentage of positive phenotypic isolates

Phenotypic tests	N	%
Positive	34	34
Negative	66	66
Total	100	100

The following table shows that of the 34 isolates of Amp-C β-lactamase, plasmid-mediated Amp-C was molecularly detected by multiplex PCR. Of these isolates, 26 isolates (76.4%) had plasmid-encoded Amp-C genes, while 8 isolates (23.6%) had no Amp-C gene.

Table 5: Positive isolates detected by Multiplex PCR

Genotypic test by Multiplex PCR	N	%
Positive	26	76.4
Negative	8	23.6
Total	34	100



Plasmid-Mediated Amp-C Gene Detection by Multiplex PCR: Agarose gel electrophoresis of nine distinct isolates' amplified Amp-C β-lactamase genes. 100 bp DNA markers are found in lane M. One Amp-C gene's amplified product is shown in lanes 1, 2, and 3. Two Amp-C genes' amplified products are shown in lanes 4, 5, 6, and 8. Lane 7 displays one Amp-C gene's amplified product. However, none of the Amp-C genes had an amplified product in the lane.

Discussion

Antibiotic resistance is a treatment challenge not only within hospital environments but also in local communities, as many bacteria have developed immunity to antibiotics [10]. This situation arises from the overuse and misuse of antibiotics in treating infectious illnesses. Antibiotic resistance is increasingly being acknowledged as a global health emergency in contemporary medical practice [3].

Klebsiella pneumoniae, classified as part of the Enterobacteriaceae family, is considered one of the most concerning pathogens associated with antibiotic resistance. As such, it has been categorised alongside other highly significant multidrug-resistant pathogens as an ESKAPE organism, which includes Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species [10].

Determining Amp-C resistance is essential for improving patient care in cases of infection and will also yield reliable data on the spread of the disease. At present, there are no established guidelines from reputable organisations for identifying Amp-C mediated resistance in gram-negative bacteria, which frequently results in misleading test outcomes, especially in phenotypic tests [11].

The prevalence of these issues in many countries remains unknown due to a lack of reliable detection methods. A number of studies have found inconsistent rates of Amp-C occurrence. Typically, high levels of Amp-C production are linked to in vitro resistance to three generations of cephalosporins and cephamycins, resulting in clinical treatment failures with broad-spectrum cephalosporins [12]. Despite numerous phenotypic tests, PCR is regarded as the gold standard procedure, but it is unfortunately not readily accessible and infrequently used in routine diagnostic laboratories [5].

100 samples of Klebsiella pneumoniae were collected from different sites of the patients in different departments of Tanta University hospital at Clinical pathology department, they were collected from patients of different ages and sex, the range of age of the patients was from (30-70) year old with Mean ± SD was (52.95 ± 8.58) by took good history from the patients there were some underlying causes as DM, Hypertension, malignancies, chronic kidney disease,

recurrent UTI and excessive antibiotic use without prescription, which was similar to the result of John *et al*. [13], while Shon *et al*. [14] in his study showed that the range of patient age was (55-60) years, males, and having mainly diabetes mellitus.

The current study found that males comprised (63%) of the isolates, whereas females made up (37%). This contrasted with the findings of Modi *et al*. [15], who reported that in their study, men accounted for (55.32%) of the cases, whereas women accounted for (44.68%). The reasons for this discrepancy were unclear, but may have been due to a greater number of male patients being tested. Conversely, studies by Maji *et al*. [16] and Ghanbari *et al*. [17] reported a higher distribution of isolates among females, which may be attributed to the fact that urinary tract infections (UTIs) are more common in females than males.

we cared about categorization of the collected isolates according to the department from which they were collected into (30%) isolates from Chest I.C.U, (24%) isolates from Emergency I.C.U, (21%) isolates from Anesthesia I.C.U, (18%) isolates from Neuropsychiatry I.C.U and (7%) isolates from Internal medicine I.C.U. the importance of this categorization was to know the rate of spread of the Klebsiella Pneumonia among these departments and incidence of the patient affection by the organism.

The (100) isolates are then categorized in this study according to the origin of the isolate into (34%) isolates from sputum, (28%) isolates from pus & wound, (17%) isolates from urine, (14%) isolate from blood, (4%) isolates from tracheal tube and (3%) isolates from bronchoalveolar lavage.

According to the Modified Kirby Bauer disc diffusion method, the isolated bacteria in the current study were found to be multi-drug resistant when antibiotic discs were applied to them. However, they also demonstrated sensitivity to specific antibiotics, such as Meropenem (46.6%), Tigecycline (80%), Imipenem (60%) and Colistin (86%). These results are similar to those of the Zorgani *et al*. research [18], which reported (95.5%) multi-drug resistance. In contrast, Abujnah *et al*. [19] found that (42%) of the isolates were multi-drug resistant. The results suggest that strains of K. pneumoniae pose a significant threat in terms of treatment and public health, necessitating the enforcement of strict hygiene practices and regular monitoring studies to identify the genetic basis of resistance.

The Amp-C E test was performed on the MDR isolates collected, revealing that 34% of the isolates were positive for the Amp-C enzyme and 66% were negative, as reported by Zorgani *et al* [18], who also found that 44.4% of isolates were phenotypically positive using the E-test.

While in the study of Blomstrom *et al*, (20) showed a higher percentage than our study (88%), these differences in the results may be due to different strains of K. pneumoniae in the communities which poses different genes from each other's.

The current research found that among the 34 isolates identified as Amp-C positive via phenotypic testing, 76.4% [26] produced the Amp-C *β*-lactamase gene as detected by multiplex PCR. The incidence rate found in this study exceeded that of Wassef *et al*. [21], who found that 26.5% of samples contained the plasmid-mediated Amp-C gene, and also surpassed the percentage of Amp-C-producing K.pneumoniae strains, which Yilmaz *et al*. [22] reported to be 3.6% in the tested population, as identified by PCR.

The difference in outcomes observed between these studies can be attributed to the fact that this study's prevalence calculation was based on the [34] isolates identified as Amp-C positive using the phenotypic method, as opposed to Yilmaz *et al*.'s analysis, which computed the prevalence using all 22 isolates. More population-based prevalence research is needed to assess the actual spread of Amp-C *β*-lactamases.

Three genes were identified in the isolates that showed positive phenotypic screening results. CMY-1 was the most prevalent type in all isolates, occurring in 65.3% of them, followed closely by CMY-2 in 50.0%, and then FOX-1 in 34.6%. A study by Fam *et al*. [23] discovered that in a Cairo, Egypt, analysis of clinical Enterobacteriaceae isolates, 28.3% of the population harbored the Amp-C gene, which included strains of E. coli, Klebsiella, and P. mirabilis. Furthermore, it was discovered that six out of ten Klebsiella species and all six E. coli bacteria that tested positive for Amp-C included the CMY-2 enzyme. While DHA-1 was found in three K. pneumoniae isolates and one P. mirabilis strain, only one K. pneumoniae sample carried the CMY-4 enzyme.

22 Amp-C genes were found in 25.8% of bacterial isolates that tested positive for cefoxitin sensitivity, according to research by Montgomery *et al*. [24]. 40.9% of them were categorised as belonging to the FOX and MOX (CMY) families, 13.6% to the EBC family, and 4.5% to the CIT family.

Our research found that CMY-1 and CMY-2 were the predominant genes in our area, whereas research by Fam *et al*. [23] identified CMY-2 and DHA-1 as the prevalent Amp-C gene cluster in our region. Earlier research has also pinpointed DHA-type enzymes in Taiwan as noted by Tan *et al*. [25] and in China as reported by Yadav *et al*. [26]. The strain CMY-2 has been characterised in Salmonella enterica serovar Typhimurium [28]. Katri *et al*. found that 29 isolates of K. pneumoniae in a Tunisian university hospital carried the CMY-4 Amp-C *β*-lactamases. The research discovered that certain isolates possessed a number of genes. Ten isolates possessed both the CMY-1 and CMY-2 genes, whereas five isolates harboured both the CMY-1 and FOX-1 genes. Furthermore, four isolates were identified as carrying three genes: CMY-1, CMY-2, and FOX-1. Wassef *et al*. [21] reported that a single isolate of Klebsiella contained three distinct genes: bla FOX, bla MOX, and bla CIT. Yusuf *et al*. [30] found that key factors contributing to infection with bacteria that produce ESBL and Amp-C include long-term antibiotic use, extended periods in intensive care, residing in a nursing home, severe illness, and living in an environment with high usage of third-generation cephalosporins and invasive medical procedures such as catheterization. Previous research on the link between antibiotic use and the risk of infections from bacteria producing Amp-C β-lactamase in adults has yielded conflicting findings: some studies have established a connection, such as Lee *et al*. [31], who found that antibiotic exposure and hospital environments are among the risk factors for acquiring resistant bacteria, whereas others have not, including Matsumura *et al*. [32], who discovered that immunosuppression and prior antibiotic use actually reduced the risk of infection from Amp-C β-lactamase producers. Research published by Zerr *et al*. [33] showed that past use of antibiotics was not a major risk factor for having bacteria resistant to ampicillin-carbénicillin compared to bacteria that were susceptible to it, however exposure to third generation cephalosporins was found to have a significant association with such resistant bacteria.

Current antimicrobial sensitivity tests are insufficient to

identify Amp-C β-lactamase mediated drug resistance affecting 3rd generation cephalosporins, which can result in treatment failure. The presence of Amp-C β-lactamases complicates the treatment of infections caused by inherently resistant bacteria, which are generally resistant to most antibiotics [34].

In the present study, depending on Amp-C E test in screening of Amp-C β lactamase, study the antimicrobial susceptibility of cefoxitin resistant K. pneumoniae showed full resistance to ampicillin, ampicillin/sulbactam, third generation cephalosporins, such as ceftriaxone, cefotaxime, cefazolin followed by high resistance to aztreonam (89%) then trimethoprim/ sulfamethoxazole (77.8%).

The fourth-generation cephalosporins are capable of rapidly crossing the outer membrane and are commonly employed to treat infections caused by bacteria that produce Amp C [35]. In this study, it was discovered that the bacteria exhibited a high level of resistance to cefepime, with a resistance rate of 77.8 percent, suggesting that fourth generation cephalosporins should not be used. Research by Liu and Liu [36] showed that highly resistant Amp-C producing Klebsiella pneumoniae had a similar susceptibility to cefepime, specifically 78.6%.

Carbapenem antibiotics are recommended for treating infections caused by Klebsiella pneumoniae strains that produce Amp-C, due to their stability against beta-lactamases and high affinity for penicillin binding proteins [37].

In this study, the most effective antibiotics identified were Colistin, Tigecycline, and carbapenems. Compared to the majority of antibiotics commonly used, these drugs are relatively pricey. The use of these substances has likely been limited, resulting in the organisms becoming vulnerable to them. Notwithstanding a relatively low incidence of imipenem resistance in our research, the danger of carbapenemase dissemination remains substantial.

Administration of parenteral antibiotics, including imipenem, necessitates hospitalization and close monitoring, resulting in significant expenses for the patient and limiting their use to secondary treatment options. As a result, the use of carbapenems should be limited to complex and longstanding cases [38].

There are currently no CLSI-recommended assays for Amp-C β-lactamase detection. Cefoxitin resistance is a reliable indicator of Amp-C synthesis. For laboratory diagnosis and confirmation of AmpC-producing Gram-negative bacteria, phenotypic techniques combined with cefoxitin as a screening tool may provide a more effective approach [39].

Phenotypic confirmation tests are inexpensive, despite being highly sensitive and specific, as demonstrated in studies conducted by reference [40]. Genotypic characterisation is widely regarded as the gold standard, but molecular techniques can be difficult to implement, necessitate highly skilled staff, and are typically more expensive [41].

Many studies have found the co-existence of Amp-C β-lactamase and ESBL, and this may be due to the plasmid-mediated Amp-C β-lactamase that has been widely disseminated among the Enterobacteriaceae. The presence of Amp-C β-lactamase alongside ESBLs can obscure phenotypic identification of the latter, as noted in reference [38].

These phenotypic methods have restricted sensitivity and specificity, and, in addition, they are incapable of distinguishing between chromosomal and plasmid-mediated Amp-C producers. Consequently, molecular tests are still necessary for accurate identification of organisms harbouring Amp-C genes on a plasmid [42].

Conclusion

- Studies have revealed that Klebsiella pneumoniae that produces Amp-C has a very high level of resistance to cephalosporins and medications in the beta-lactam class. The most sensitive antibiotics, tigecycline, colistin, and carbapenems, such imipenem and meropenem, should only be used as such. Since they were the most sensitive antibiotics, tigecycline, colistin, and carbapenems—more especially, imipenem and meropenem—should be used as backup drugs. The most sensitive antibiotics, such as tigecycline, colistin, and carbapenems like imipenem and meropenem, should be retained as reserved medications.
- Identifying different types of Ampicillin-Cloxacillin may help hospitals to manage infection control more effectively and enable doctors to prescribe the most suitable and potent antibiotic, thereby reducing the selective pressure that contributes to the development of antibiotic resistance. Determining the different types of Ampicillin-Carbencillin (Amp-C) can help hospitals control infections and enable doctors to prescribe the most suitable and effective antibiotic, ultimately reducing the selective pressure that leads to antibiotic resistance.
- To better understand the strains' genetic relationships and molecular epidemiology in relation to this resistance, it could be necessary to ascertain the strains' order and type. To further understand the strains' genetic relatedness and the molecular epidemiology of this resistance, it could be required to sequence and type them. To learn more about the molecular epidemiology and genetic relatedness of this resistance, the strains may need to be sequenced and typed.

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Potential Conflict of Interest

No conflicts of interest have been declared.

Authors' Contributions

All authors contributed equally to the design, work, statistical analysis, and manuscript preparation. All contributing authors have endorsed the final draft of the article.

References

1. Rameshwari T, Atul K, Varsha C, Paramjit S, Agarwal N. Prevalence of various beta-lactamase among gram negative bacilli in urinary isolates from patients in a tertiary care hospital of northern India. Prevalence. 2017;10(4):129-132.
2. Singhal S, Mathur T, Khan S, Upadhyay DJ, Chugh S, *et al*. Evaluation of methods for AmpC β-lactamase in gram negative clinical isolates from tertiary care hospitals. Indian J Med Microbiol. 2005;23(2):120-124.
3. El-Hady SA, Adel LA. Occurrence and detection of AmpC β-lactamases among Enterobacteriaceae isolates from patients at Ain Shams University Hospital. Egyptian J Med Hum Genet. 2015;16(3):239-244.
4. Gupta G, Tak V, Mathur P. Detection of AmpC β-lactamases in gram-negative bacteria. J Lab Physicians. 2014;6(1):1-6.
5. Smith M, Diederen B, Scharringa J, Leversteijn-van

Hall M, Fluit AC, *et al*. Rapid and accurate detection of carbapenemase genes in Enterobacteriaceae with the Cepheid Xpert Carba-R assay. J Med Microbiol. 2016;65(9):951-953.
6. Mohamudha PR, Harish BN, Parija SC. Molecular description of plasmid-mediated AmpC β-lactamases among nosocomial isolates of Escherichia coli & Klebsiella pneumoniae from six different hospitals in India. Indian J Med Res. 2012;135(1):114-119.
7. Cheesebrough M. District laboratory practice in tropical countries part 2. 2nd ed. New York: Cambridge University Press; c2007.
8. Black JA, Moland ES, Thomson KS. AmpC disk test for detection of plasmid-mediated AmpC β-lactamases in Enterobacteriaceae lacking chromosomal AmpC β-lactamases. J Clin Microbiol. 2005;43(6):3110-3113.
9. Pérez-Pérez FJ, Hanson ND. Detection of plasmid mediated AmpC β-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol. 2002;40(6):2153-2162.
10. Drawz SM, Bonomo RA, Burghoffer B. Three decades of β-lactamase inhibitors. Clin Microbiol Rev. 2010;23(2):160-201.
11. Handa D, Pandey A, Asthana A, Rawat A, Handa S, Thakuria B. Evaluation of phenotypic tests for the detection of AmpC betalactamase in clinical isolates of Escherichia coli. Indian J Pathol Microbiol. 2013;56(2):135-138.
12. Karadiya R, Hooja S, Pal N, Sharma R, Maheshwari R, *et al*. Prevalence and antimicrobial susceptibility of ESBL and AmpC β-lactamase producing Escherichia coli and Klebsiella pneumoniae from various clinical samples: an emerging threat. J Evol Med Dent Sci. 2016;5(32):1729-1734.
13. John MS, Meenakshi K, Munilakshmi P, Reddy PS. Prevalence and Distribution of Bacterial Pathogens Causing Urinary Tract Infections in Humans: A Study from Tertiary Care Hospital in AP, India. Int J Curr Microbiol Appl Sci. 2015;4(2):251-257.
14. John MS, Meenakshi K, Munilakshmi P, Reddy PS. Prevalence and Distribution of Bacterial Pathogens Causing Urinary Tract Infections in Humans: A Study from Tertiary Care Hospital in AP, India. Int J Curr Microbiol Appl Sci. 2013;4(2):251-257.
15. Modi PS, Kumar A, Jethava SD, Sanghavi RR. Bacterial prevalence, antibiotic sensitivity pattern, predisposing factors, nosocomial UTI. Bacterial prevalence, antibiotic sensitivity pattern and predisposing factors in patients of nosocomial urinary tract infection (UTI) visited the tertiary care hospital in Jamnagar region, Western Gujarat, India. J Evid Based Med Healthc. 2017;4(62):3746-3751.
16. Maji SK, Mandal PK, Panja C, Dolai TK, Samanta A, *et al*. Prevalence and antibacterial susceptibility pattern of aerobic bacteria causing urinary tract infection in tribal population in rural part of West Bengal, India. Int J Curr Microbiol Appl Sci. 2016;5(6):406-412.
17. Ghanbari F, Khademi F, Saberianpour S, Shahin M, Ghanbari N, *et al*. An Epidemiological Study on the Prevalence and Antibiotic Resistance Patterns of Bacteria Isolated from Urinary Tract Infections in Central Iran. Avicenna J Clin Microbiol Infect. 2017;4(3):7-12.
18. Zorgani HD, Sufya N, Bashein A, Elahmer O, Chouchani C. Open Microbiol J. 2017;11:195-202.
19. Abujnah AA, Zorgani A, Sabri MA. Multidrug resistance and extended-spectrum β-lactamases genes among Escherichia coli from patients with urinary tract infections in Northwestern Libya. Libyan J Med. 2015;10:26412.
20. Bolmström A, Engelhardt A, Bylund L, Ho P, Karlsson A. Evaluation of two new etest strips for AmpC detection, abstr. D-0451. Abstr 46th Intersci Conf Antimicrob Agents Chemother; c2009.
21. Wassef M, Behiry I, Younan M, El Guindy N, Mostafa S, *et al*. Genotypic identification of AmpC β-lactamases production in gram-negative bacilli isolates. Jundishapur J Microbiol. 2014;7(1):1-8.
22. Yilmaz NO, Agus N, Bozcal E, Oner O, Uzel A. Detection of plasmid-mediated AmpC β-lactamase in Escherichia coli and Klebsiella pneumoniae. Indian J Med Microbiol. 2013;31(1):53-59.
23. Fam N, Gamal D, El Said M, Abo-Fadi L, El Dabei E, *et al*. Detection of Plasmid-Mediated AmpC Beta-Lactamases in Clinically Significant Bacterial Isolates in a Research Institute Hospital in Egypt. Life Sci J. 2013;10(2):2294-2304.
24. Montgomery J, Nakos J, Gurtler V. Comparison of phenotypic tests for detection of plasmid-mediated AmpC β-lactamases. In: ESCMID 18th European Congress of Clinical Microbiology and Infectious Diseases; 2008 Apr 19-22; Barcelona, Spain.
25. Tan TY, He J, Yong Ng LS, Koh TH, Hsu LY. Evaluation of Screening Methods to Detect Plasmid-Mediated AmpC in Escherichia coli, Klebsiella pneumoniae and Proteus mirabilis. Antimicrob Agents Chemother. 2009;53(1):146-149.
26. Yadav K, Prakash S. Antimicrobial Resistance Pattern of Uropathogens Causing Urinary Tract Infection (UTI) Among Diabetics. Biomed Res Int. 2016;1:07-15.
27. Al-Agamy M. Genetic basis of cefotaxime resistant isolates of Klebsiella pneumoniae from Cairo. Afr J Microbiol Res. 2016;6(1):20-27.
28. Ahmed A, Shimamoto T. Molecular characterization of antimicrobial resistance in Gram-negative bacteria isolated from bovine mastitis in Egypt. Microbiol Immunol. 2011;55:318-327.
29. Ktari S, Arlet G, Mnif B, Gautier V, Mahjoubi F, Ben Jmeaa M. Emergence of multidrug-resistant Klebsiella pneumoniae isolates producing VIM-4 Metallo-β-Lactamase, CTX-M-15 extended spectrum β-lactamase, and CMY-4 AmpC β-Lactamase in a Tunisian University Hospital. Antimicrob Agents Chemother. 2006;50(12):4198-4201.
30. Yusuf I, Haruna M, Yahaya H. Prevalence and antibiotic susceptibility of AmpC producing clinical isolates at a tertiary healthcare center in Kano, Northwest Nigeria. Afr J Microbiol Res. 2013;14(2):109-119.
31. Lee CH, Lee YT, Kung CH, Ku WW, Kuo SC, *et al*. Risk factors of community-onset urinary tract infections caused by plasmid-mediated AmpC beta-lactamase-producing Enterobacteriaceae. J Microbiol Immunol Infect. 2015;48(3):269-275.
32. Matsumura Y, Nagao M, Iguchi M, Yagi T, Komori T, *et al*. Molecular and clinical characterization of plasmid-mediated AmpC beta-lactamase producing Escherichia coli bacteraemia: a comparison with extended spectrum beta-lactamase-producing and non-resistant E. coli bacteraemia. Clin Microbiol Infect. 2013;19(2):161-168.

33. Zerr DM, Miles-Jay A, Kronman MP, Zhou C, Adler AL, *et al*. Previous antibiotic exposure increases risk of infection with extended-spectrum-β-lactamase- and AmpC-producing Escherichia coli and Klebsiella pneumoniae in pediatric patients. Antimicrob Agents Chemother. 2016;60(7):4237-4243.
34. Karim F, Rashid H, Sattar ANI, Aluned S, Saleh AA, *et al*. Phenotypic Detection of AmpC B-Lactamases in Gram-Negative Bacteria. Bangladesh J Med Microbiol. 2016;2(1):28-32.
35. Shi WF, Zhou J, Qin JP. Trans-conjugation and genotyping of the plasmid mediated AmpC beta lactamase and extended spectrum beta lactamase genes in Klebsiella pneumoniae. Chin Med J. 2009;122(9):1092-1096.
36. Liu XQ, Liu YR. Detection and genotype analysis of AmpC β lactamase in Klebsiella pneumoniae from tertiary hospitals. Exp Ther Med. 2016;12(1):480-484.
37. Queenan AM, Bush K. Carbapenemases: the versatile β-lactamases. Clin Microbiol Rev. 2007;20(3):440-458.
38. Kaur S, Awari A. Study of Uropathogenic Multidrug Resistant Escherichia coli and Klebsiella Species with Reference to Extended Spectrum Beta Lactamase and Amp C Beta Lactamase. Int J Curr Microbiol Appl Sci. 2016;5(6):784-789.
39. Manoharan A, Sugumar M, Kumar A, Jose H, Mathai D, ICMR-ESBL study group. Phenotypic & molecular characterization of AmpC β-lactamases among Escherichia coli, Klebsiella spp., & Enterobacter spp. from five Indian Medical Centers. Indian J Med Res. 2012;135(3):359-364.
40. Peter-Getzlaff S, Polsfuss S, Poledica M, Hombach M, Giger J, *et al*. Detection of AmpC Beta-Lactamase in Escherichia coli: Comparison of Three Phenotypic Confirmation Assays and Genetic Analysis. J Clin Microbiol. 2011;49(8):2924-2932.
41. Ashok AK, Jaryal SC, Thakur K, Sood A, Gupta PK, *et al*. Detection of Inducible and Non-inducible (constitutive) AmpC β-lactamase-producing Gram-Negative Bacteria among Family Enterobacteriaceae by Two Phenotypic Methods-Disk Antagonism Test (DAT) and AmpC disk Test at a Tertiary Care Hospital, Himachal Pradesh, India. Int J Curr Microbiol Appl Sci. 2016;5(4):133-139.
42. Saffar H, Niaraki NA, Tali AG, Baseri Z, Abdollahi A, *et al*. Prevalence of AmpC β-lactamase in Clinical Isolates of Escherichia coli, Klebsiella spp., and Proteus mirabilis in a Tertiary Hospital in Tehran, Iran. Jundishapur J Microbiol. 2016;9(12):1-11.

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