Molecular Detection of Per-1 Type Extended Spectrum \( β \)-Lactamase among Clinical Isolates of Klebsiella Pneumoniae in Baghdad Hospitals

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Abstract

This study was aimed to determine the presence of PER-1 type Extended-Spectrum \( β \)-Lactamases (ESBLs) in clinical isolates of Klebsiella pneumonia in Baghdad. During a period extended for three months (July-September, 2014), 26 clinical isolates were collected from two hospitals in Baghdad (Fatima-Al-Zahra hospital for pediatric and obstetric and Baghdad Teaching Hospital). Out of these 26 isolates, 11 isolates from blood, 7 from urine, 6 from sputum and 2 from wound. All isolates had 100% resistance to ampicillin and ceftazolin while the most effective antibiotics were imipenem and meropenem with high sensitivity rate (92.31%). Out of 26 K. pneumoniae isolates, 17(65.38%) isolates were positive ESBL producing isolates phenotypes and 9(34.62%) isolates were negative ESBL producing isolates (non ESBLs). Genotypic Detection of blaPER-1 gene by polymerase chain reaction showed that out of all seventeen ESBL-producing K. Pneumoniae isolates by phenotypic detection, 6 isolates (13.8%) were contained blaPER-1 gene (925bp).

Keywords: Klebsiella pneumonia, Blaper-1 gene, Extended-spectrum \( β \)-lactamases (ESBLs).

Introduction

Klebsiella pneumoniae is a member of Enterobactericeae [1]. It is one of important nosocomial pathogens, which causing urinary tract infections, pneumonia, blood and wound infections [2].

Extended-spectrum cephalosporin's, such as those of fourth-generation (cefpime) and third-generation (ceftriaxone, cefotaxime and ceftazidime), are mostly used as antibiotics for the treatment of severe infections, because of their low toxicity, strong bactericidal activity and ample spectrum [3].

During the past few decades, an increasing in prevalence and incidence of diseases caused by number of Extended-spectrum cephalosporin's resistant Klebsiella pneumonia isolates have been reported worldwide [3-4]. Extended-spectrum \( β \) -lactamases (ESBLs) are plasmid-mediated enzymes which can hydrolyze monobactama and broad spectrum \( β \)-lactams, they are transmitted by plasmids among bacterial strains [5].

The PER-1 type Extended-spectrum beta \( β \) – lactamase is a class \( A \) enzyme which conferring high-level of resistance to antibiotics of \( β \)-lactams [6-7]. The purpose of this study was to determine the presence of PER-1 type ESBLs in clinical isolates of Klebsiella pneumonia isolated from several hospitals in Baghdad.

Materials and Methods

Bacterial Isolates

Twenty six isolates of Klebsiella pneumonia were collected from two hospitals in Baghdad through a period extended for three months (July-September, 2014). Identification of the isolates at species level was done by using Vitek-2 system (Bio-Merieux, France), by using ID-GNB cards according to the manufacturer's instructions.

Antibiotic Susceptibility Test

Resistance to ceftazidime and other different antibiotics was done by using Vitek-2 system (Bio-Merieux, France), by AST cards according to the manufacturer's instructions. Minimum inhibitory concentrations (MICs) for ceftazidime were also determined by Vitek-2 system (Bio-Merieux, France) using the AST cards.

Phenotypic Detection of ESBL

Phenotypic detection of the ESBL producing isolates was done to all isolates by Vitek-2 system (Bio-Merieux, France), using the AST cards and by NCCLS confirmatory test [8], in which the organism was swabbed on to a Mueller-Hinton agar plate. Ceftazidime (30 \( μ \)g) and ceftazidime...
plus clavulanic acid (30/10 μg) were placed on Mueller-Hinton agar and incubated. Organism was considered as ESBL producer, if there was a 5mm increase in zone diameter of ceftazidime / clavulanate disc and that of ceftazidime disc alone.

Genotypic Detection of BlaPER-1 Gene

Genotypic Detection of blaPER-1 gene was done by polymerase chain reaction (PCR) to all isolates which showing ESBL positive by phenotypic detection (by Vitek-2 system and double-disk method).

DNA Extraction

DNA was obtained by suspending 2-3 colonies of each test isolate grown on MacConkey agar plates in 500 mL of nuclease-free water (Promega, USA) and heating at 90°C for 10 min. using a water bath. Samples were spun at 10000 rpm for 10 min. These samples were used as the bacterial DNA template for PCR assay [9].

Amplification Reaction

PCR mixture was set up in a total volume of 25 μl (for each isolate) included: 12.5μl of Master mix 2X (Promega, USA), nuclease-free water (3.5μl), DNA sample (5μl), blaPER1 Forwarded primer: 5’- ATGAAT GTCATTATAAAAGC -3’ (2μl) and Reverse primer: 5’- AATTTGGGCTTAG GGCAGAA -3’ (2μl) (Alpha DNA, Canada) . Temperature cycling conditions included an initial denaturation at 94°C for 5 min followed by 31 cycles of 94°C for 45 seconds, annealing at 45°C for 30 seconds and extension at 72°C for 30 seconds. Cycling was followed by a final extension at 72°C for 7 min [10].

Agarose Gel Electrophoresis

Gel electrophoresis was used for the detection of PCR products which visualized with the aid of ethidium bromide and UV transilluminat or documentation system [11].

Results

Bacterial Isolates

Twenty six clinical isolates of K. pneumoniae have been collected from two hospitals in Baghdad (Fattima-Al-Zahra hospital for pediatric and obstetric and Baghdad Teaching Hospital), these isolates were yielded from different specimens included blood (n=11), urine (n=7), sputum (n=6) and wound (n=2). High percentage of K. pneumoniae isolation was from blood specimens (42.31%) followed by urine specimens (26.92%) and sputum specimens (23.07%), while the low percentage of isolation was in wound and burn specimens (7.7%).

Antibiotic Susceptibility Test

Susceptibility of all 26 K. pneumonia isolates toward 16 different antibiotics; ampicillin, cefazolin, ampicillin / sulbactam, amoxicillin / clavulanic acid, cefepime, ceftazidime, ceftriaxion, levofloxacin, ciprofloxacin, imipenem, meropenem, gentamicin, piperacillin/tazobactam, tobramycin, nitrofurantion, and trimethoprim/sulphamethoxazole were tested by Vitek-2 system using AST cards.

Data presented in figure-1 shows a high level of resistance of K. pneumoniae clinical isolates to most of the antibiotics under test. Figure-1 shows that all K. pneumoniae clinical isolates had 100% resistance to ampicillin and cefazolin, followed by trimethoprim/ sulphamethoxazole which gave 25 (96.15%) resistance rates.

The most effective antibiotics were imipenem and meropenem which showed high sensitivity rate 24 (92.31%), followed by piperacillin/tazobactam, levofloxacin and ciprofloxacin which all showed 20 (76.92%) sensitivity rate. Other antibiotics showed different degree of resistance as shown in Figure-1.
producing isolates while 9(34.62%) isolates were negative ESBL producing isolates (Figure-2).

**Phenotypic detection of ESBL**

Out of 26 Klebsiella pneumoniae isolates, 17(65.38%) isolates were positive ESBL producing isolates while 9(34.62%) isolates were negative ESBL producing isolates (Figure-2).

All 17 K. Pneumoniae ESBL producing isolates gave positive result for ESBL Screening by both Vitek-2 system and double-disk method (Figure-3).

**Genotypic Detection of Blaper-1 Gene**

In this study, all seventeen ESBL-producing K. pneumoniae isolates were subjected to PCR to detect blaPER-1 gene. Among all K. pneumoniae producing ESBLs isolates, 6(13.8%) isolates were contained blaPER-1 gene as shown in Figure-4.

**Figure 2:** Number of positive and negative ESBL producing K. pneumonia isolates.

**Figure 3:** NCCLS Confirmatory Test for detection ESBL producers Klebsiella pneumoniae isolates.

CAZ= Ceftazidine

CEC= ceftazidime/clavulanic acid

**Figure 4:** Electrophoresis to detect blaPER-1 PCR product was done on agarose gel (1%) in 0.5X TBE buffer using 75 V. for 1 hrs.; Lanes: 1, 2, 3, 4, 5 and 14 were 925bp of blaPER-1 PCR products; Lanes: 6, 7, 8, 9, 10, 11, 12, 13, 15, 16 and 17 were negative PCR product; Lane M: Kappa 100 bp DNA ladder; lane C: Negative control (had all PCR mixture including water instead of DNA template).
In this study, the PER-1 extended-spectrum beta-lactamase was found in 6(13.8%) ESBL-producing K. Pneumonia isolates under the study. In a study done by [13], found that blaPER-1-positive strains consisted of P. aeruginosa, A. baumannii and Alcaligenesfaecalis. Also blaPER-1 was not observed in any of the examined E. coli, K. pneumonia and Stenotrophomonasmaltophilia isolates.

Discussion

In this study, we obtained 17(65.38%) positive ESBL phenotypes isolates of K. pneumoniae while negative (non-ESBL) phenotypes were in 9(34.62%) isolates of K. pneumoniae. Malhotra et al. [12] reported that K. pneumonia and Eschericia coli are amongst the important ESBL producing gram negative bacilli.

References


