

Spreading of NDMI-Producing *Klebsiella Pneumoniae* in Different Wards at Assiut University Hospital

Eman Ramadan Mohamed^{1,*}, Hamada Mohamed Halby², Mamdouh Yones Ali³,
Rehab Mahmoud Abd El-Baky⁴, Nancy G F M Waly⁵

¹Department of Microbiology and Immunology, Faculty of Pharmacy, Al-Azhar University, Assiut71515, Egypt

²Department of Microbiology and Immunology, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt

³Department of Microbiology and Immunology, Faculty of Pharmacy, Al-Azhar University, Minia, 61519, Egypt

⁴Department of Microbiology and Immunology, Faculty of Pharmacy, Minia University, Minia, 61519, Deraya University, Minia 11566, Egypt

⁵Department of Microbiology and Immunology, Faculty of Pharmacy, Minia University, Minia, Egypt

*Corresponding author: emanabelbary@azhar.edu.eg

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Abstract Spreading of NDMI-producing *klebsiella pneumoniae* has become a great trouble in many Egyptian hospitalized patients. Localizing the source of these isolates is an important step to prevent their spread in this country. This study included 33 NDMI-producing *klebsiella pneumoniae* that collected from different clinical specimens of patients admitted to different wards at Assiut University Hospitals in Egypt and confirmed to produce blaNDM1 gene by polymerase chain reaction. Isolates were typed by Enterobacterial Repetitive Intergenic Consensus (ERIC). Respiratory samples (51%) were the predominant samples and chest department was the major ward (48.48%) that isolates recovered. ERIC gel profile showed that several identical isolates were found especially in chest ward and between different medical wards of this hospital suggested clonal transmission of NDMI-producing *K. pneumoniae*. Also, there are differences in the number and size of ERIC-PCR profiles indicated the genetic diversity among NDMI-producing *K. pneumoniae* isolates. The results of this study may help in tracing and controlling NDM-1-producing *K. pneumoniae* outbreak by applying effective infection control measures especially in the chest ward of this hospital.

Keywords: carbapenem-resistant *Klebsiella pneumoniae*, blaNDM1, ERIC

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1. Introduction

K. pneumoniae has become a great challenge for infection control in human health that can cause life-threatening several infections, such as blood stream infection, pneumonia, urinary tract, post-surgical, and intensive care-related infections [1]. Such infections result in increasing morbidity, mortality and medical hospital costs [2].

K. pneumoniae can resist carbapenem drugs by numerous mechanisms, Production of carbapenemases is the most mechanism [3]. New Delhi-metallo beta-lactamase (NDM) is the most important novel metallo-beta-lactamase that can hydrolyze to nearly all β -lactams [3,4]. It was firstly reported in a Swedish patient traveled to New Delhi [5]. Then, worldwide spread of NDM-1-producing isolates have also reported [6]. However, NDM1-producing *K. pneumoniae* is emerged in numerous Egyptian hospital [7,8,9] study on dissemination mechanism of blaNDM1 gene by clonal similarity is limited.

Typing for distinguishing bacterial isolates of the same species are essential epidemiological tools in tracing the source of infection [10]. Enterobacterial Repetitive Intergenic Consensus (ERIC) is considered molecular typing tool used in determining the clonal transfer of *K. pneumoniae* infection in many hospital settings [11,12]. This study is performed to help in tracing and stopping dissemination of NDM1-producing *K. pneumoniae* in our hospital.

2. Material and Methods

2.1. Study Design

This study is a hospital based observational study, conducted for a period of 14 months from April 2018 to May 2019 on 126 *Klebsiella pneumoniae* isolates obtained from different clinical specimens of 126 patients admitted to different wards at Assiut University Hospital in Egypt.

2.2. Sample Collection

Samples were collected in a sterile container from each patient according to sites of infections.; they included blood, peritoneal fluid, urine, respiratory secretions, wound swabs, and cerebrospinal fluid. Only one isolate per patient was included. They were transported to Infection Control Lab. within 2 hours of collection. Viable bacterial count was done for urine samples [13] and blood culture was done for blood samples [14]. Quantitative culture of endotracheal aspirate (EA) [15].

2.3. Isolation and Identification of *K. Pneumoniae*

Bacterial identification to the species level were carried out by colonial morphology on MacConkey's agar plates (Oxoid, UK), Gram stained films, biochemical reactions including oxidase, motility, indole, methyl red, voges-proskauer, citrate and urease tests and confirmed with API 20E (BioMerieux, Inc., Hazelwood, MO).

2.4. DNA Isolation

Klebsiella isolates were cultured in Luria-Broth medium (Oxoid, Hampshire, England) overnight at 37 °C. DNA isolation of each isolate was done using Genomic Purification Kit (Fermentas, Lithuania) based on the manufacturer's protocol.

2.5 Detection of Carbapenem Resistant *K. Pneumoniae*

Modified carbapenem inactivation methods (mCIM) were performed as described by byPierce et al [16] for Suspected Carbapenemase-Producing *K. pneumoniae* isolates. EDTA-modified carbapenem inactivation method (eCIM) for detection of MβL enzymes [17].

2.6. Detection of *Bla NDM1* Gene

The gene *bla* NDM-1 was amplified using primers and conditions as described by [18]. The sequences of primers used is F (GGTTTGGCGATCTGGTTTTC-3') and R(CGGAATGGCTCATCACGATC-3'). A volume of 2μL of template DNA was added to a final volume of 25 μL PCR mixture comprising 12.5 μL of Taq PCR Master Mix (Fermentas, UK), including, 1× PCR buffer, 1.5 mmol/L MgCl₂, 0.15 mmol/L dNTP, and 1.25 IU Taq DNA polymerase, 1 μL of 0.8 μmol/L each primer and 9.5 μL of sterile distilled water. The amplicon size was 621 bp and was analyzed by electrophoresis in a 1.5% agarose gel.

2.7. ERIC-PCR

ERIC-PCR was applied using the primers, ERIC1 (5'-ATGTAAGCTCCTGGGGATTAC-3') and ERIC2 (5'-AAGTAAGTACTGGGGTGAGCG-3') for *K. pneumoniae* isolates [19]. PCR Amplifications were performed in a 25 μL solution under the following conditions: an initial denaturation at 94°C for 5 min; 1 min at 94°C, 1 min at 39°C, and 5 min at 72°C; and a final extension at 72°C for 10 min. Denaturation, annealing,

and extension were performed in 35 cycles. ERIC-PCR products were detected by a 2.0% agarose gel electrophoresis, and the gel was visualized with UV. Products sizes were estimated using 100 bp and 1 kb DNA ladders (New England Biolabs) as molecular size markers.

2.8. DNA Fingerprinting

Analyses of the DNA fingerprints were presented by BioNumerics software version 7.6 (Applied Maths, Belgium). A cutoff value similarity was applied to define identical strains as closely related isolates with ≥95% similarity and isolates with <95% similarity as unrelated strains. The similarity between the profiles was evaluated with the band matching Dice coefficient, and dendrogram was produced by the unweighted pair group method with arithmetic averages (UPGMA).

3. Results

3.1. Identification of *K. Pneumoniae* Isolates

K. pneumoniae which identified by conventional biochemical tests was confirmed and fully investigated by using the API 20E system. The API20E Index system identified *Klebsiella Pneumoniae* with two different analytical profile index numbers with 5215773 code and 1215773 code.

3.2. Patients and Isolates Characteristics

One hundred and twenty-six *Klebsiella pneumoniae* strains isolated from different departments at Assiut University Hospital (April 2018 to May 2019) were tested for carbapenemase enzymes production using mCIM and eCIM. Carbapenemase activity was found in 77/126 (61.1%) of the *K. pneumoniae* isolates. *Bla*NDM1 was positive in 43/77 (55.84%) isolates.

This study included 33 patients out of 43 with confirmed NDMI-producing *K. pneumoniae* infections. The isolates were obtained from 19 male and 14 female patients. Patients were generally elderly (median age was 50 years, with a range of 40–63 years). Respiratory samples (51%) were the predominant sources of KP, followed by urine (21%), wound (16%), blood (6%), and catheter tip (6%) samples.

3.3. The Different Medical Wards from which the 33 NDMI-Producing Isolates were Recovered

Table 1. Prevalence of *K. pneumoniae* in Different Hospital Wards.

Medical wards	No. n=33	%
Neurology	1	3.03
Surgery	2	6.06
Chest	16	48.48
Ped. ICU	3	9.09
Injuries	2	6.06
GIT	6	18.18
Critical situations	1	3.03
Burns	1	3.03
General reception	1	3.03

GIT:Gastrointestinal tract, Ped. ICU:Pediatric Intensive care unit.

Mostly of Medical Wards from which the clinical Klebsiella isolates recovered were Chest Department (48.48%) and Gastrointestinal tract wards (18.18%) as presented in Table 1.

3.4. Production of *Bla* NDMI Gene

K. pneumoniae isolates were confirmed to produce NDMI gene by PCR method. Amplicon size was 621bp as appeared in Figure 1.

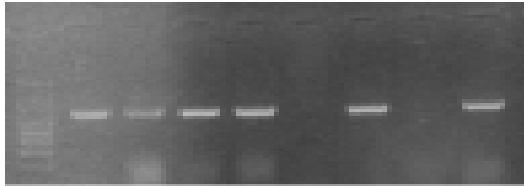


Figure 1. Gel electrophoresis of the PCR-amplified products for detection of *bla_{NDMI}*. Lane M is DNA marker (100-1500bp); lane 1 is a positive control, lanes 2,3,4,6,8 are positive samples (621 bp) and lane 5,7 are negative samples for *bla_{NDMI}*

3.5. ERIC profiles of NDMI-Producing *K. Pneumoniae*

All 33 NDMI-producing *K. pneumoniae* isolates were

genotyped by ERIC that showed greater genetic diversity of the isolates (Figure 2). The electrophoretic analysis of the PCR reaction products has revealed that the number of bands ranged from 1 to 5. The sizes of the PCR products ranged from 100 bp to more than 1000 bp.

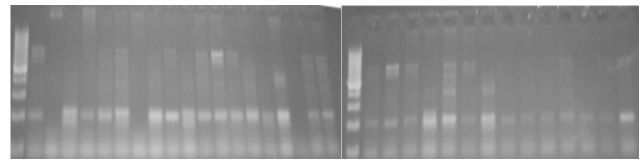


Figure 2. ERIC profiles of 33 NDMI-producing *K. pneumoniae*. Lane L is DNA marker (100-1000bp).

3.6. DNA Fingerprinting

By analyzing the ERIC-PCR profiles, the 33 isolates were categorized into 25 ERIC-types. Isolates sharing greater or equal to 95% of the bands were classified in the same clusters as presented in the dendrogram (Figure 3). ERIC identified fifteen (15) types of *K. pneumoniae* that were designated as follows: type A (n=4), type B (n=3) and two isolates in each type from C to type E. The remaining unrelated (n=20) isolates with <95% similarity that belonged to types F1 to F 20 had one isolates in each type as shown in Table 2.

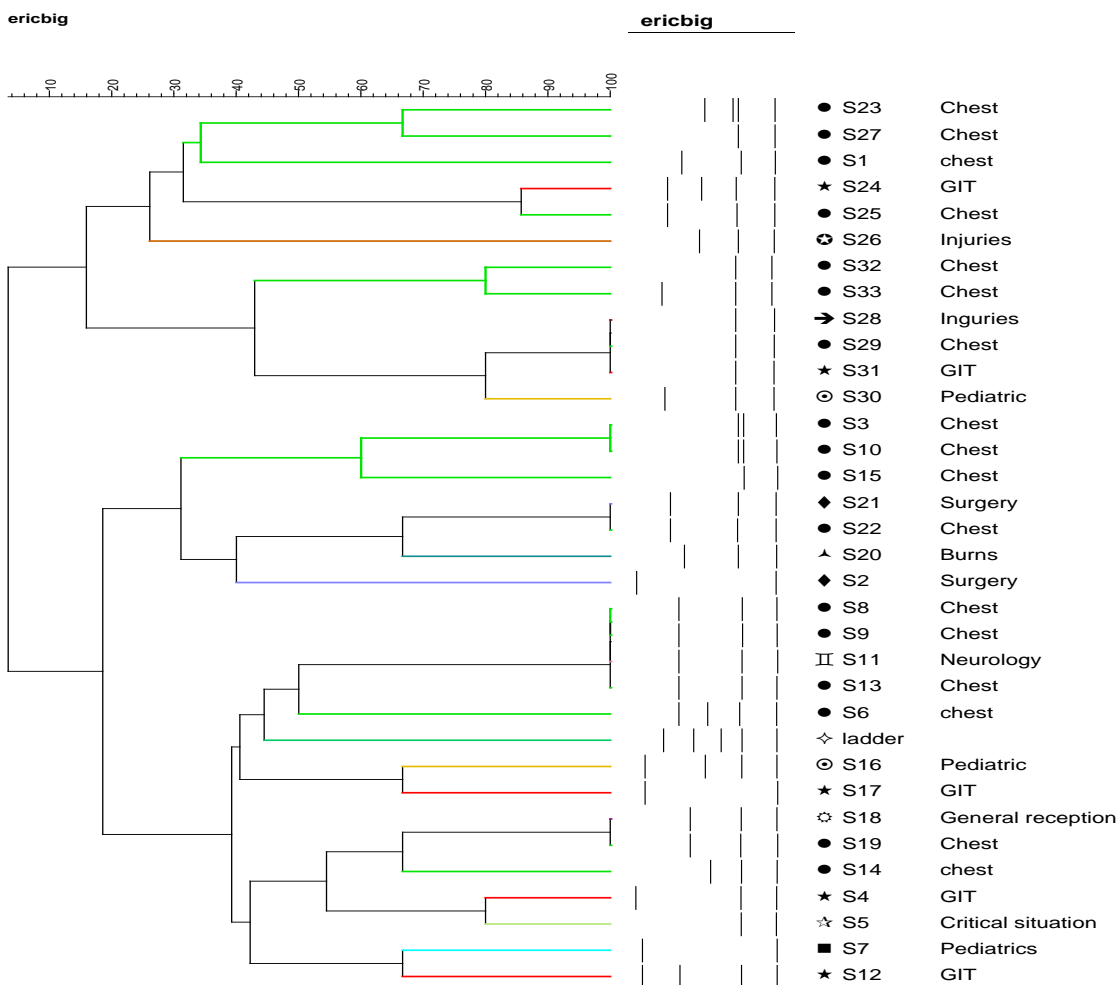


Figure 3. Dendrogram comparing ERIC profiles of NDMI-producing *K. pneumoniae* isolates

Table 2. ERIC Profiles of NDM1-producing *K. pneumoniae*.

ERIC Type	Strains
A	8,9,11,13
B	28,29,31
C	3,10
D	18,19
E	21,22
F1 to F20	Remaining unrelated isolates

4. Discussion

K. pneumoniae is known as the main organisms that cause nosocomial infections including bacteremia, pneumonia, urinary tract infections, intra-abdominal infections, sepsis and gastroenteritis especially in developing countries [1,2]. It can easily acquire antibiotic resistant causing a global challenge to human health [20]. carbapenem resistant *K. pneumoniae* has become more problem in limiting of treatment option [21,22]. It can produce *bla*NDM1 gene to hydrolyze carbapenem drugs [23]. There was a spread of carbapenem resistant *K. pneumoniae* in many hospitals of our region during the last few years [7,8,9]. Additionally, it was approved previously that different clones of *K. pneumoniae* can spread easily in one ward (PICU) of this hospital [24]. As a result, a need for localizing the source and controlling the spread of NDM1-producing *K. pneumoniae* in different wards within this hospital should be employed. PCR-based ERIC fingerprinting is a quick method useful for typing the strains of *K. Pneumoniae* [11].

In this study, Respiratory samples (51%) were the predominant samples and Chest Department was the major ward (48.48%) that isolates recovered. This may be the airway is the most common presentations of *K. pneumoniae* in hospitals [25].

By imagining of the ERIC gel profiles, several identical isolates were found suggested clonal transmission of *K. pneumoniae* in different medical wards of this hospital [26]. Also, there are differences in the number and size of ERIC-PCR profiles indicated the genetic diversity among NDM1-producing *K. pneumoniae* isolates.

In this study, most of ERIC profiles exhibited in type A (S8, S9, S11, S13). This clone spread dramatically in chest ward that proved *K. pneumoniae* can spread easily by respiratory droplet. Additionally, this clone appeared (S11) in neurology department that may indicate transfer of *bla*NDM1 gene to another ward by this clone. Another clone (type C) can spread between two patients in chest ward presented in isolates 3 and 10.

ERIC showed that three of NDM1-producing *K. pneumoniae* isolates (S28, S29, S31) from injury, chest and GIT departments belonged to the same cluster (type B) which suggested that this clone that harbor NDM1 can spread between patients in different wards indicating that easily spread of hospital acquired NDM1-producing *K. pneumoniae*.

Similar strains were detected between two isolates (S18, S19) from general reception and chest (type D) and similarity was also detected (type E) between 2 isolates (S21, S22) from surgery and chest wards, demonstrated that chest ward could be the common factor of spread of

the carbapenem resistant of *K. pneumoniae* to other wards of this hospital and may be the origin of spreading [27].

However, genetic analysis using ERIC-PCR showed that majority of NDM1-producing isolates (n=20) were clonally unrelated, suggesting that dissemination of NDM1 genemay due to possible horizontal transmission between non similar patterns by conjugative plasmid [28,29].

The results of this study represent a need to additional precaution to rationalize carbapenem drugs, develop hygiene measures and effective infection control measures to control this NDM-1-producing *K. pneumoniae* outbreak especially in the chest ward of this hospital.

5. Conclusion

Highly resistant of NDM1- producing *K. pneumoniae* should be traced and controlled especially in developing country. More typing methods are needed for estimation of the genetic relatedness and tracing the sources of infection to other hospitals by this pathogen.

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Conflict of Interest

The authors have no competing interests.

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