Overview of Klebsiella Pneumoniae as a Nosocomial Pathogen and ESBL Producing Strains in Iran

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Abstract: Klebsiella pneumoniae is one of the most important human bacterial pathogens with an extensive range of community and hospital acquired infections that may lead to morbidity and mortality. Evaluating the prevalence and epidemic sources of infections and the pathogenicity mechanism of bacteria can be investigated by various typing methods. The emergence of multi-drug resistant strains and extended-spectrum β-lactamase (ESBL) producing isolates has already become a great challenge in nosocomial infection incidence. There are several reports on ESBL isolates of K. pneumoniae in Iran. However, our aim is a comprehensive analysis on ESBL isolates K. pneumoniae from different parts of Iran which has not yet been performed.

Keywords: Klebsiella Pneumonia, Nosocomial, Infection, ESBL

Introduction

Klebsiella pneumoniae is one of the most abundant species of the Klebsiella genus that causes complications such as urinary tract infections, Ventilator-Associated Pneumonia (VAP), sepsis and endophthalmitis in Asia and America. Emergence of multi-drug resistant strains has already become a great challenge in nosocomial infection incidence (Pokra et al., 2016; Kashani and Elliott, 2013). In 1883, Friedlander, the German microbiologist and pathologist, isolated the encapsulated bacilli from a patient with pneumonia. The bacterium was initially called Friedlander's bacillus but was renamed Klebsiella due to Edwin Klebs. Currently, the Klebsiella genus is classified among the five predominant common gram negative pathogens that could lead to nosocomial infections (Horan et al., 1988). Klebsiella oxytoca, Klebsiella rhinoscleromatis and Klebsiella ozaenae are the main subspecies of K. pneumoniae based on nucleic acid hybridization (Sakazaki et al., 1989). In addition, Klebsiella terrigena, Klebsiella ornithinolytica, Klebsiella planticola and Klebsiella aerogenes are known other species (Izard et al., 1981; Gavini et al., 1986; Iyer et al., 2017). Nowadays, more than 50% of these strains are isolated from wound, respiratory and urinary tract infections (Podschun and Ullmann, 1994). There are several reports on ESBL isolates of K. pneumoniae in Iran. However, a comprehensive analysis of ESBL isolates of K. pneumoniae from different parts of Iran has not yet been performed. The searches were done according to several English and Persian databases including PubMed, Scopus, Isi, Iranmedex and SID to identify studies addressing ESBL isolates of K. pneumoniae in Iran during the past decade.

Genomic Structure

In Holt and colleagues’ study, more than 300 isolates of Klebsiella pneumoniae strains were investigated based on whole-genome sequencing method that lead to KpI (K. pneumoniae), KpII (K. quasipneumoniae) and KpIII (K. variicola) as the three main distinct species, of which K. pneumoniae is the most significant one in human infections (Fig. 1). The most important gene clusters are associated with various virulence factors, regulators of mucoid phenotype (rmpA, rmpA2), siderophore systems, the ferric uptake operon kfuABC, the two-component regulator kvgAS and an allantoinase gene cluster. The three chromosomal core genes are classified as LEN β-lactamases, SHV and OKP. On the other hand both FosA and oqxA/B that associate in resistance to fosfomycin and quinolones have been transferred horizontally from Escherichia coli (Holt et al., 2015; Chen et al., 2014).

Cell Structure, Metabolism and Natural Habitat

The most vital metabolic pathways of K. pneumoniae are recapitulated as glycolysis, tricarboxylic acid, oxidation of fatty acids and creatine phosphate (Dong et al., 2012). Moreover K. pneumoniae is able to produce 2-butanol from glucose in 2-3 butanediol synthesis process (Chen et al., 2015).
**Microbiology and Epidemiology**

*K. pneumoniae* belonging to the family Enterobacteriaceae is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod-shaped bacterium that can grow in potassium cyanide citrate with no growth at 10°C (Goetz *et al.*, 1995). The main source of clinical infections is gastrointestinal tract infection and also hospital staff's hands, though the most outbreaks are found on neonatal wards (Montgomerie, 1979).

**Pathogenic Factors**

The most important virulence factors are as follows:

- **Capsule:** *K. pneumoniae* capsule structure which consists of repeating sugar units (4-6) which completely including uronic acid residues. This polysaccharide capsule has ability for attachment and producing biofilm formation, however, has
enough capacity to provide resistance to desiccation, preserves from phagocytosis against polymorphonuclears and granulocytes and also from serum bactericidal effect and activation of the C3b complement (Magill et al., 2014; March et al., 2013). On the other hand, strains with repetitive sequences of mannose-a-2/3-mannose or l-rhamnose-a-2/3-l-rhamnose have less pathogenicity than others, until now 78 various capsular serotypes were defined (Hsu et al., 2013)

- **Fimbriae**: *K. pneumoniae* has type 1 and 3 of pili, in which type 1 pili mediates hemagglutination of guinea pig erythrocytes, has the ability to interact with D-mannose residues of glycoprotein receptors on host cells and salivary and genital membrane surfaces (Gupta et al., 2003; Firon et al., 1984), whereas type 3 pili has capacity to mannose-resistant agglutination of human erythrocytes which were treated with tannic acid. This type of pilus consists of MrkA (original) and MrkD (adhesion) subunits that depends on mrkABCDF operon and responsible for biofilm formation, binding to tracheal epithelium, renal and lung tissue cells (Babu et al., 1986; Ares et al., 2017). Ares et al. conducted a study that confirms the essential role of H-NS protein in the regulation of type 3 polysaccharide capsule of *K. pneumoniae* (Ares et al., 2017)

- **Outer Membrane Proteins (OMP)**: These proteins have a critical function in materials transport and pathogenicity. The role of OmpA as a eukaryotic cell adhesion, serum resistance and protects the bacteria against galectin-3 is noticeable (Ares et al., 2016). Moreover, OmpK35 and OmpK36 have been reported as a two main outer membrane porins, which are homologous as OmpF and OmpC. It should be mentioned that both OmpK35 and OmpK36 are related with extended-spectrum β-lactamase and associated with carbapenem resistance strains in *K. pneumoniae* respectively (Llubet et al., 2009; Tsai et al., 2011)

- **Phospholipase activity**: Leroy et al. study indicates the role of phospholipase (Dpld1) as a new virulence factor in *K. pneumoniae* (Lery et al., 2014)

- **Siderophore**: these high-affinity iron-chelating compounds which were secreted by many microorganisms are required for bacterial growth, reproduction and spread of infection especially during pneumonia inflammation and bacterial dissemination. This event depends on the activation of the master transcription factor hypoxia inducible factor-1 (HIF-1) protein and also inducing cytokine secretion (Holden et al., 2016)

### Typing Methods

Evaluating the prevalence and epidemic sources of infections and the pathogenicity mechanism of bacteria can be investigated by various typing methods, like PFGE, MLST, RAPD, Rep-PCR and etc:

- **Pulsed-field gel electrophoresis (PFGE)**: is one of the most common techniques for identifying the epidemiological and nosocomial source infections (Holden et al., 2016; de Souza Lopes et al., 2005)

- **Multilocus Sequence Typing (MLST)**: This molecular technique has designed based on the study of DNA housekeeping genes and their alleles (Cuzon et al., 2010)

- **RAPD**: In this molecular technique the short random sequences of the bacterial genome are amplified by oligonucleotide primers (Holden et al., 2016)

- **Rep-PCR**: In this technique short repetitive sequences of bacteria are analyzed by oligonucleotide primers. This method is based on DNA fingerprinting techniques (Siu et al., 2011; Nielsen et al., 2011)

- **MALDI-TOF Mass Spectrometry**: matrix-assisted laser desorption/ionization- time-of-flight mass spectrometer is used for microbial identification, bacterial typing, epidemiological studies and also evaluation of antibiotic resistant strains (Perez et al., 2010)

- **Conventional methods**: serotyping, phage typing and bacteriocin typing are the most common methods and are used as the best typing for this bacteria (Berrazeg et al., 2013; Slopek et al., 1967; Rennie and Duncan, 1974)

### Antibiotic Resistance

*K. pneumoniae* is naturally resistant against several antibiotic agents such as penicillin, ampicillin, amoxicillin, oxacillin, carbencillin due to frequency of β-lactamase genes (Orskov and Orskov, 1984; da Silva et al., 2012; Chambers, 2000). Resistance to β-lactamase and carbapenem antibiotics is associated through a range of β-lactamase, such as strains SHV, TEM, CTX-M and carbapenemase respectively (Chaves et al., 2001). Strains which are harboring SHV-1 and TEM-1 may be resistant to piperacillin or first-generation cephalosporin (Grundmann et al., 2010; Girlich et al., 2000; Lemozy et al., 1995). Moreover, ESBL producing strains were reported for the first time in Germany that are responsible for resistance to cephalosporins such as ceftaxime, ceftriaxone and cefotaxime and monobactams (aztreonam) (Nicolas-Chanoine, 1997; Knothe et al., 1983). Due to this issue the prevalence of antibiotic-resistant A. baumannii strains have increased in Iran and this may cause significant clinical problems. In addition, the AmpC gene was also identified in *K. pneumoniae* strains, albeit in another form called MIR-1, which is 90% similar to Enterobacter cloacae. This gene contains FOX-1, FOX-2, FOX-3, CMI-2, CMI-4, CMI-8, MOX-1, MOX-2, DHA-1, DHA-2, LAT-1, LAT-2 and
ACC-1 (Jacoby and Sutton, 1991; Philippou et al., 2002). These strains are resistant to aminopenicillins, carbapenems, and cephalosporins; while these classes of genes are not well able to hydrolyse with cefepime or a carbapenem. Relevant studies that were performed in different regions of Iran are described in Table 1.

**Treatment**

As shown in Table 1, reported Klebsiella resistance rates in Iran ranged as high as 96% and as seen in Fig. 2, mean multilab drug resistance rates generally increased over time and the last set of isolates collected in Iran were more resistant to all antibiotics (30%). The highest rates of resistance were observed towards β-lactam antibiotics (ceftriaxone, cefotaxime, piperacillin, ceftazidime, cefepime, amikacin, and ampicillin). Also, most of the isolates from all over the country were still sensitive to imipenem, meropenem, tazocin, piperacillin-tazobactam and amikacin and the imipenem is still a effective drug in Iran.

**Prevention and Control**

According to conducted studies, identifying the risk factors and mechanisms of drug resistance is related to various enzymes that are produced, including ESBLs, MBLs, KPC and Amp-C belonging to Ambler A, B and C groups. Identification of these resistance factors will lead to the pivotal proper treatment. Direct contact limitation between patients and healthy people, following patients under treatment and compliance with individual health are the critical strategies for controlling the outbreak infections.

### Table 1: The prevalence of antibiotic-resistant ESBL producing strains in the different regions of Iran

<table>
<thead>
<tr>
<th>Authors</th>
<th>The percentage of ESBL producing strains</th>
<th>Genes</th>
<th>The Highest rate of antibiotic resistance</th>
<th>enrolment time</th>
<th>Type of sample</th>
<th>Province</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shahcheraghi et al. (2007)</td>
<td>33%</td>
<td>-</td>
<td>Carbapenem, Piperacillin, Cefotaxime and Ceftriaxone</td>
<td>2006</td>
<td>urine, blood, wounds, sputum</td>
<td>Tehran</td>
<td>(Shahcheraghi et al. 2007)</td>
</tr>
<tr>
<td>Aminzadeh et al. (2008)</td>
<td>52.3%</td>
<td>-</td>
<td>Amoxicillin, Cephalexin, and Cefotaxime</td>
<td>2007</td>
<td>urine</td>
<td>Tehran</td>
<td>(Aminzadeh et al., 2008)</td>
</tr>
<tr>
<td>Behroozi et al. (2010)</td>
<td>12%</td>
<td>-</td>
<td>Ceftazidime, Cefotaxime, Amoxicillin, Carbapenem, and Ceftriaxone</td>
<td>2009</td>
<td>urine</td>
<td>Tehran</td>
<td>(Behroozi et al., 2010)</td>
</tr>
<tr>
<td>Naabi et al. (2010)</td>
<td>96%</td>
<td>blaCTX-M (20%), blaTEM (2.6%), blaOXA (45.14%), blaPER (35.1%)</td>
<td>Cefotaxime, Ceftriaxone, and Piperacillin</td>
<td>2006-2007</td>
<td>urine, blood, wound, sputum, CSF, central venous line and intra-abdominal abscesses</td>
<td>from different three general and two private hospitals in Iran</td>
<td>(Naabi et al., 2010)</td>
</tr>
<tr>
<td>Mansouri et al. (2012)</td>
<td>41.3%</td>
<td>blaTEM (20%), blaOXA (18%), blaPER (7.5%)</td>
<td>Aminocillin, Cephalaxin, Ceftriaxone and Gentamicin</td>
<td>2007-2008</td>
<td>urine</td>
<td>Tehran</td>
<td>(Mansouri et al., 2012)</td>
</tr>
<tr>
<td>Eftekhar et al. (2012)</td>
<td>27.43%</td>
<td>blaCTX-M (20%), blaOXA (45.14%), blaPER (35.1%)</td>
<td>Ceftriaxone, Cefotaxime and Piperacillin</td>
<td>2008</td>
<td>urine</td>
<td>Tehran</td>
<td>(Eftekhar et al., 2012)</td>
</tr>
<tr>
<td>Riahi Zamani et al. (2012)</td>
<td>20%</td>
<td>blaOXA (8.77%), blaPER (0.52%)</td>
<td>-</td>
<td>2009-2010</td>
<td>urine, sputum, and wound aspirates, peritonitis and pulmonary infections</td>
<td>Mashhad</td>
<td>(Riahi Zamani et al., 2012)</td>
</tr>
<tr>
<td>Khosravi et al. (2013)</td>
<td>47.27%</td>
<td>blaCTX-M (46.15%), blaOXA (14.15%), blaPER (40.64%), blaOXA (38.17%), blaPER (44.28%)</td>
<td>Amoxicillin, Cephalaxin, Ceftriaxone, and Gentamicin</td>
<td>2012</td>
<td>-</td>
<td>Alborz</td>
<td>(Khosravi et al., 2013)</td>
</tr>
<tr>
<td>Astini et al. (2014)</td>
<td></td>
<td>-</td>
<td>Ceftriaxone, Cefotaxime, and Gentamicin</td>
<td>2011</td>
<td>from patients a burn unit</td>
<td>Tehran</td>
<td>(Astini et al., 2014)</td>
</tr>
<tr>
<td>Derakhshan et al. (2014)</td>
<td>54.9%</td>
<td>blaOXA (38.9%)</td>
<td>Ceftriaxone</td>
<td>2011</td>
<td>urine, blood, and wound aspirates and other samples (including catheter, eye and etc.).</td>
<td>Tehran</td>
<td>(Derakhshan et al., 2014)</td>
</tr>
<tr>
<td>Hashemi et al. (2014)</td>
<td>57.3%</td>
<td>blaOXA (9%), blaOXA (62.3%), blaOXA (38.17%), blaPER (46.28%)</td>
<td>Amoxicillin, Cefpodoxime, Ceftriaxone, and Piperacillin</td>
<td>2012</td>
<td>urine, blood, culture, sputum, body fluids, and other samples</td>
<td>Tehran</td>
<td>(Hashemi et al., 2014)</td>
</tr>
<tr>
<td>Ghaliopour et al. (2014)</td>
<td>38.18%</td>
<td>blaOXA (41.3%), blaPER (44.28%)</td>
<td>Ceftriaxone, Amoxicillin, and Gentamicin</td>
<td>2012</td>
<td>-</td>
<td>Isfahan</td>
<td>(Ghaliopour et al., 2014)</td>
</tr>
<tr>
<td>Riasi et al. (2014)</td>
<td>46.9%</td>
<td>blaOXA (57.5%), blaPER (68.98%)</td>
<td>Ceftriaxone, Cefotaxime, and Gentamicin</td>
<td>2008-2012</td>
<td>urine, blood, and body fluids</td>
<td>Tehran</td>
<td>(Riasi et al., 2014)</td>
</tr>
<tr>
<td>Mansouri et al. (2014)</td>
<td>28%</td>
<td>blaOXA (46.15%), blaPER (35.1%)</td>
<td>Ceftriaxone, Cefotaxime, and Gentamicin</td>
<td>2007-2008</td>
<td>blood, urine and body fluids</td>
<td>Kerman</td>
<td>(Mansouri et al., 2014)</td>
</tr>
<tr>
<td>Inadli et al. (2014)</td>
<td>43%</td>
<td>blaOXA (38.17%), blaPER (46.28%)</td>
<td>Ceftriaxone, Cefotaxime, and Gentamicin</td>
<td>2011-2012</td>
<td>urine, wound, blood</td>
<td>Mashhad</td>
<td>(Inadli et al., 2014)</td>
</tr>
<tr>
<td>Saeidi et al. (2014)</td>
<td>66.6%</td>
<td>blaOXA (65%), blaPER (65%)</td>
<td>Ceftriaxone, Cefotaxime, and Gentamicin</td>
<td>2010-2011</td>
<td>urine culture</td>
<td>Zabol</td>
<td>(Saeidi et al., 2014)</td>
</tr>
<tr>
<td>Fathi et al. (2015)</td>
<td>-</td>
<td>blaOXA (41.3%)</td>
<td>Piperacillin, Amoxicillin, and Ceftriaxone</td>
<td>2012-2013</td>
<td>urine, wound, breast aspirate, bronchoalveolar-lavage fluid, sputum, and wound aspirate</td>
<td>Isfahan</td>
<td>(Fathi et al., 2015)</td>
</tr>
<tr>
<td>Rajbouini et al. (2015)</td>
<td>-</td>
<td>blaOXA (30%), blaPER (22.2%), blaOXA (19%), blaPER (16%)</td>
<td>Imipenem, Ceftriaxone, Trimethoprim-sulfamethoxazole, and Cefotaxime</td>
<td>2015</td>
<td>urine, wound, body fluids, blood, sputum, and other samples</td>
<td>Babol</td>
<td>(Rajbouini et al., 2015)</td>
</tr>
<tr>
<td>Mansourizadeh et al. (2016)</td>
<td>-</td>
<td>blaOXA (30%), blaPER (22.2%), blaOXA (19%), blaPER (16%)</td>
<td>Imipenem, Ceftriaxone, Trimethoprim-sulfamethoxazole, and Cefotaxime</td>
<td>2012-2013</td>
<td>from urine, sputum, wound, blood, and other samples</td>
<td>Shiraz</td>
<td>(Mansourizadeh et al., 2016)</td>
</tr>
<tr>
<td>Maleki et al. (2018)</td>
<td>25.5%</td>
<td>blaOXA (92%), blaCTX-M (78%)</td>
<td>Ceftriaxone and Cefotaxime</td>
<td>2013</td>
<td>urine</td>
<td>Isfahan</td>
<td>(Maleki et al., 2018)</td>
</tr>
</tbody>
</table>
Loss of this porin may be one of the factors contributing to antimicrobial resistance among ESBL-producing K. pneumoniae and may favor the selection of additional mechanisms of resistance. Microbiology laboratories must be able to identify resistant bacteria in a timely suitable manner, especially those that are falsely susceptible in vitro to antibiotics. Bacteriological excellence is needed more than ever (57).

**Conclusion**

There is a relatively high prevalence of drug resistant K. pneumoniae isolates in Iran. This review showed that the prevalence of ESBL-producing K. pneumoniae varies in different regions of Iran and the capital city of Iran (Tehran,) has a higher incidence of ESBL compared to northern regions and the western cities. Thus, a high degree of awareness among physicians and microbiologists, active infection control committees, appropriate antimicrobial therapy, improvement of hygiene conditions and monitoring of drug resistant isolates are urgently needed in order to better control the emergence and spread of ESBL K. pneumonia isolates in hospital settings.

**Ethical Consideration**

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely addressed by the authors.

**Conflict of Interests**

The authors declare that there is no conflict of interests.

**References**


