Diagnostic Microbiology and Infectious Disease 80 (2014) 267–271

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Comparison of multilocus sequence typing, RAPD, and MALDI-TOF mass spectrometry for typing of β-lactam–resistant *Klebsiella pneumoniae* strains

Svea Sachse [a], Stephanie Bresan [a], Marcel Erhard [b], Birgit Edel [a], Wolfgang Pfister [a], Angela Saupe [a], Jürgen Rödel [a,*]

[a] Institute of Medical Microbiology, University Hospital of Jena, D-07747 Jena, Germany
[b] RIPAC-LABOR, D-14476 Potsdam, Germany

ARTICLE INFO

Article history:
Received 31 January 2014
Received in revised form 20 June 2014
Accepted 8 September 2014
Available online 16 September 2014

Keywords:
Klebsiella
ESBL
Typing
MALDI-TOF
RAPD
MLST

ABSTRACT

Extended spectrum of β-lactam (ESBL) resistance of *Klebsiella pneumoniae* has become an increasing problem in hospital infections. Typing of isolates is important to establish the intrahospital surveillance of resistant clones. In this study, the discriminatory potential of randomly amplified polymorphic DNA and matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) analyses were compared with multilocus sequence typing (MLST) by using 17 β-lactam–resistant *K. pneumoniae* isolates of different genotypes. MLST alleles were distributed in 8 sequence types (STs). Among ESBL strains of the same ST, the presence of different β-lactamase genes was common. RAPD band patterns also revealed 8 types that corresponded to MLST-defined genotypes in 15 out of 17 cases. MALDI-TOF analysis could differentiate 5 clusters of strains. The results of this work show that RAPD may be usable as a rapid screening method for the intrahospital surveillance of *K. pneumoniae*, allowing a discrimination of clonally related strains. MALDI-TOF-based typing was not strongly corresponding to genotyping and warrants further investigation.

1. Introduction

Klebsiella pneumoniae is an important nosocomial pathogen that is adapted to the hospital environment (Podschun and Ullmann, 1998). It is one on the most frequent extended-spectrum β-lactamase (ESBL) producers and can become multidrug resistant by acquisition of carbapenemases and selection of additional resistances against quinolones (Pfeifer et al., 2010). The rapid dissemination of resistant strains is responsible for ongoing outbreaks of multidrug-resistant *Klebsiella* strains worldwide, including those harboring KPC, VIM, or OXA-48 carbapenemases (Kitchel et al., 2010; Steinmann et al., 2011; Voulgari et al., 2013). Patients hospitalized for a prolonged time exhibit an increased infection risk from antibiotic resistant *Klebsiella* strains (Steinmann et al., 2011). To prevent an ongoing dissemination in a hospital setting, typing of isolates is important to establish the surveillance of resistant clones.

At present, multilocus sequence typing (MLST) has been established as the reference technique to examine the clonal identity of bacterial strains (Diancourt et al., 2005). Defining sequence types included in a database offers the opportunity for large scale interhospital surveillance. However, there is also a need for less expensive and laborious methods in routine diagnosis that allow rapid evaluation of the relatedness of strains, on a local scale, to identify outbreaks with multiresistant *K. pneumoniae*. Previously, randomly amplified polymorphic DNA (RAPD) analysis has been established as a fast genetic fingerprinting method that is much less laborious than pulsed-field gel electrophoresis (Deschaght et al., 2011). Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) approaches now used for identification of bacteria may also offer the potential to evaluate the identity of strains within 1 species (Berrazeg et al., 2013; Novais et al., 2014; Schaumann et al., 2012; Trevino et al., 2011). Here, we describe the epidemiology of ESBL and carbapenem-resistant *K. pneumoniae* strains isolated within a 5-month period at the University Hospital of Jena, Germany. The aim of the study was to evaluate the discriminating potential of MLST, RAPD, and standard MALDI-TOF analysis for studying the relatedness of *K. pneumoniae* isolates.

2. Materials and methods

2.1. Bacterial strains and susceptibility testing

The study included 17 *K. pneumoniae* strains with third-generation cephalosporin resistance collected from patients of intensive care and surgical units between December 2011 and April 2012. Strains were cultured from samples on Columbia sheep blood agar and Drigalski lactose agar. Species identification was performed with Vitek-2

* Corresponding author. Tel.: +49-36419393633; fax: +49-36419393502.
E-mail address: juergen.roedel@med.uni-jena.de (J. Rödel).

http://dx.doi.org/10.1016/j.diagmicrobio.2014.09.005
0732-8893/

(bioMerieux, Nürtingen, Germany) and subsequently confirmed by MALDI-TOF mass spectrometry (MS, AXIMA Confidence; Shimadzu Europe, Duisburg, Germany). MICs of aminopenicillins, cephalosporins, and carbapenems were measured using Vitek-2. ESBL resistance was confirmed by the combination disk method using cefotaxim (CTX) versus CTX + clavulanic acid (CV) and cefpodoxime (CPD) versus CPD + CV. Meropenem and imipenem MICs were verified by E-test on Mueller–Hinton agar and interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST)-defined breakpoints.

2.2. Detection of β-lactamase genes

ESBL genes were detected by the Identibac AMR-ve DNA microarray (Alere, Jena, Germany). Amplification, hybridization, and array scanning were performed according the manufacturer's protocol. Data were analyzed using IconoClust software (Alere). Lack or presence of a carbapenemase was examined with a modified Hodge test and by PCR (HyplexSuperBug, Amplex Bio Systems, Giessen, Germany) detecting VIM, IMP, OXA-48, KPC, and NDM-1. Carbapenemase PCR was performed when an increased meropenem MIC was measured (≥2 mg/L).

2.3. RAPD

RAPD analysis was performed according to the protocol published by Deschaght et al. (2011) using primer RAPD4 (AAGACGCCGT). 25 ng (2 μL) of DNA was added to 12.5 μL multiplex mastermix (Qiagen, Hilden, Germany), 1 μL primer (10 pmol), and 9.5 μL Diethylpyrocarbonate (DEPC)-treated H_2O. PCR consisted of an initial incubation at 94 °C for 15 min and then cycling for 45 times at 94 °C for 1 min, 37 °C for 1 min, and 72 °C for 2 min. RAPD fragments were visualized by agarose gel electrophoresis.

2.4. MLST

Sequence typing was performed according to the paper published by Diancourt et al. (2005). The method uses primers for the amplification and sequencing of internal portions of 7 house-keeping genes of *K. pneumoniae*, including *rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *infB*, and *tonB* (Diancourt et al., 2005). Cycle sequence PCR was performed using the BigDyeTeminator Cycle Sequencing Kit v1.1 (Life Technologies, Darmstadt, Germany) according to the supplier's protocol. Sequences were analyzed with an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Life Technologies). Sequence types (STs) were determined using the public database of the Institute Pasteur Web site, France (www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html).

2.5. MALDI-TOF

Samples were prepared from bacterial strains grown on Columbia sheep blood agar plates for 24 h using a standard direct smear method and protein extraction on target. The mass spectra were acquired with an AXIMA Confidence MALDI-TOF MS (Shimadzu Europe) using α-cyano-4-hydroxycinnamic acid matrix and a mass range m/z from 3000 to 20000. Shimadzu Biotech Launchpad software (Shimadzu Europe) was used for spectra acquisition and peak detection. All peaks (average of 150–200) were analyzed with a threshold offset >0.015 mV. To discriminate between different *Klebsiella* strains, cluster analysis of mass spectra was performed with SARAMIS software (database V 3.10, Vitek MS Plus; bioMerieux, Nürtingen, Germany) using the

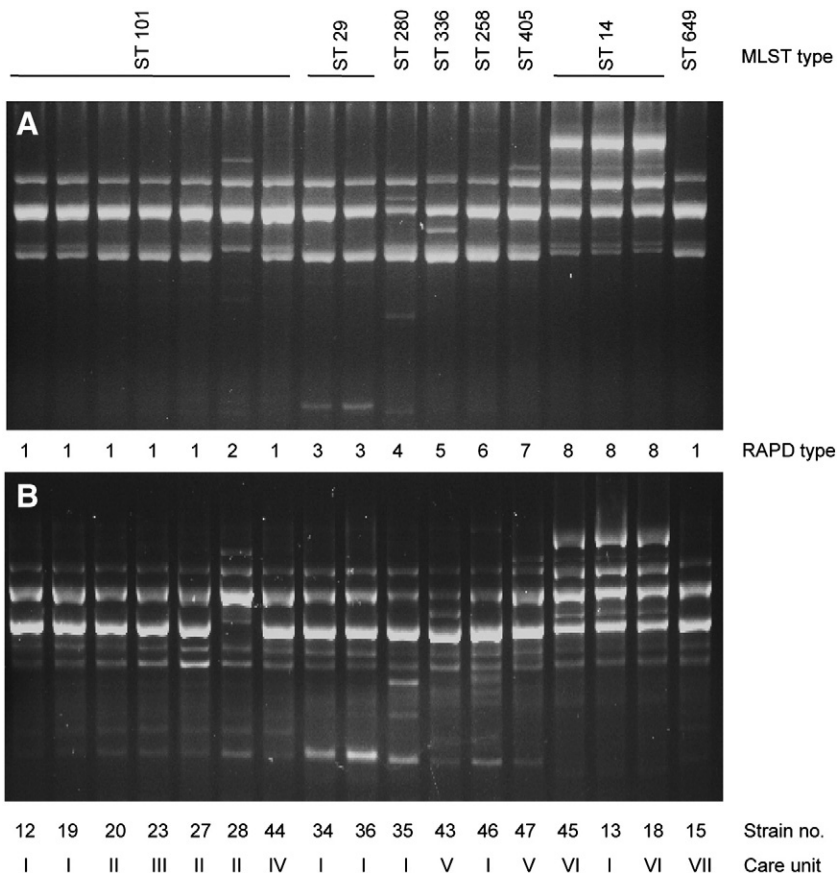

Fig. 1. MLST allelic profiles and RAPD patterns of *K. pneumoniae* isolates. The isolate number corresponds to its number in the laboratory's strain collection. RAPD analysis was performed in duplicate (A and B) to show the reproducibility of band patterns.