Cluster-distinguishing genotypic and phenotypic diversity of carbapenem-resistant Gram-negative bacteria in solid-organ transplantation patients: a comparative study

Theodoros Karampatakis,1,2 Anastasia Geladari,1 Lida Politi,3 Charalampos Antachopoulos,1,4 Elias Iosifidis,1,4 Olga Tsiatsiou,1,4 Aggeliki Karyoti,2,4 Vasileios Papanikolaou,5 Athanassios Tsakris3 and Emmanuel Roilides1,4,*

Abstract

Purpose. Solid-organ transplant recipients may display high rates of colonization and/or infection by multidrug-resistant bacteria. We analysed and compared the phenotypic and genotypic diversity of carbapenem-resistant (CR) strains of Klebsiella pneumoniae, Pseudomonas aeruginosa and Acinetobacter baumannii isolated from patients in the Solid Organ Transplantation department of our hospital.

Methodology. Between March 2012 and August 2013, 56 CR strains from various biological fluids underwent antimicrobial susceptibility testing with VITEK 2, molecular analysis by PCR amplification and genotypic analysis with pulsed-field gel electrophoresis (PFGE). They were clustered according to antimicrobial drug susceptibility and genotypic profiles. Diversity analyses were performed by calculating Simpson’s diversity index and applying computed rarefaction curves.

Results/Key findings. Among K. pneumoniae, KP-producers predominated (57.1%). VIM and OXA-23 carbapenemases prevailed among P. aeruginosa and A. baumannii (89.4 and 88.9 %, respectively). KPC-producing K. pneumoniae and OXA-23 A. baumannii were assigned in single PFGE pulsotypes. VIM-producing P. aeruginosa generated multiple pulsotypes. CR K. pneumoniae strains displayed phenotypic diversity in tigecycline, colistin (CS), amikacin (AMK), gentamicin (GEN) and co-trimoxazole (SXT) (16 clusters); P. aeruginosa displayed phenotypic diversity in cefepime (FEP), ceftazidime, aztreonam, piperacillin, piperacillin–tazobactam, AMK, GEN and CS (9 clusters); and A. baumannii displayed phenotypic diversity in AMK, GEN, SXT, FEP, tobramycin and rifampicin (8 clusters). The Simpson diversity indices for the interpretative phenotype and PFGE analysis were 0.89 and 0.6, respectively, for K. pneumoniae strains (P<0.001); 0.77 and 0.6 for P. aeruginosa (P=0.22); and 0.86 and 0.19 for A. baumannii (P=0.004).

Conclusion. The presence of different antimicrobial susceptibility profiles does not preclude the possibility that two CR K. pneumoniae or A. baumannii isolates are clonally related.

INTRODUCTION

The development and spread of antimicrobial resistance has a significant impact on patients’ mortality, and also on the length of stay and financial costs for their hospitalization, and presents a never-ending threat for clinicians and health-care systems [1, 2]. Patients receiving solid-organ transplants are quite vulnerable to colonization and infection caused by multidrug-resistant (MDR) bacteria because of their immunocompromised status and prolonged hospitalization. Colonization with MDR bacteria prior to solid-
organ transplantation (SOT), often leading to infection, has a negative effect on the transplant recipient's outcome, affecting the 60-day survival rate in liver transplant recipients [3]. The implementation of strict infection control measures may diminish the spread of MDR bacterial strains in SOT departments [4].

In Greece, as in other Mediterranean countries, carbapenem-resistant (CR) strains of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are common causes of difficult-to-treat nosocomial infections in hospitalized and critically ill patients, leading to the revival of older antimicrobials such as colistin, as there is a long route to be followed before new agents become available on the market [5, 6]. These bacteria can acquire and transmit resistance through different mechanisms. Carbapenemase production is the most frequent mechanism [7, 8]. On the other hand, many studies highlight porin loss as the predominant mechanism of resistance in CR *P. aeruginosa* strains [9].

Carbapenem resistance has become endemic in Greece [10]. However, endemic strains still cause outbreaks that affect many inpatients at the same time and may spread through medical departments. These CR strains present monoclonal or polyclonal dissemination, depending on their genotypic diversity [11, 12]. The former is very important, as monoclonal spread, which fosters horizontal transmission, emphasizes the need for the implementation of infection control measures. Moreover, they can produce diverse antimicrobial resistance phenotypes to a variable extent [12].

The aims of our study were to describe the phenotypic and genotypic patterns; to compare the phenotypic and genotypic diversity of CR *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* strains isolated in the SOT department of our hospital; and also to assess the ability of interpretative phenotype and pulsed-field gel electrophoresis (PFGE) analysis to check strain relatedness.

**METHODS**

**Bacterial isolates**

This study was part of an intervention project targeting endemic CR Gram-negative bacteria in the SOT Department of Aristotle University at Hippokration General Hospital, in which liver and kidney transplantations are performed. The project consisted of active surveillance followed by strict implementation of infection control measures to reduce infection and colonization rates. Between March 2012 and August 2013, CR strains of *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* isolated from inpatients hospitalized in the SOT department were collected for phenotypic and molecular analyses. Carbapenem resistance was defined as resistance to any carbapenem for *K. pneumoniae*, and resistance to imipenem or meropenem for *P. aeruginosa* and *A. baumannii*. These strains originated from various biological specimens (blood, urine, bronchial fluid, surgical sites, etc.) from patients with confirmed/presumed infection, as well as from weekly rectal swabs cultured on MacConkey agar plates supplemented with 1 mg L⁻¹ meropenem (MEM) obtained for active surveillance purposes. The distinction between carriage and infection was established using previously described criteria [4]. There were no repeated cultures, meaning that CR strains of the same species isolated in the same patient from both rectal swabs and biological specimens within 25 days and expressing the same phenotype were considered to be identical and were only studied once.

**Antimicrobial susceptibility testing – phenotypic analysis**

Identification and antimicrobial susceptibility testing of CR strains were performed with the VITEK 2 automated system (bioMérieux, Marcy-l’Étoile, France). The minimum inhibitory concentration (MIC) and interpretation results, expressed as sensitivity, intermediate sensitivity and resistance (SIR), were determined according to the Clinical and Laboratory Standards Institute (CLSI) breakpoints of January 2015 [13]. For *P. aeruginosa* strains, the colistin (CS) MICs were interpreted according to the clinical breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), as updated in January 2016 [14]. Tigecycline (TGC) was evaluated using the susceptibility breakpoints approved by the US Food and Drug Administration (FDA) [15]. Isolates tested within a dilution of a breakpoint were evaluated in duplicate.

CR strains were phenotypically tested for metallo-beta-lactamase (MBL) production using ethylenediaminetetra-acetic acid (EDTA) as previously described [16]. CR *K. pneumoniae* strains were further detected for *K. pneumoniae* carbapenemase (KPC) production using phenylboronic acid (PBA) as previously described [17].

**Molecular and genotypic analysis**

CR strains were screened for genes producing extended spectrum beta-lactamases (ESBL) (*bla*TEM, *bla*CTX-M, *bla*SHV, *bla*IMI, *bla*GES and *bla*PER), MBLs (*bla*VIM, *bla*IMP and *bla*SDM), KPC (*bla*KPC) and oxacillinases (*bla*OXA-48, *bla*OXA-58, *bla*OXA-23 and *bla*OXA-51). Screening was performed through PCR amplification using previously described primers and conditions [18–20].

For genotypic analysis of CR *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* strains, DNA was digested using the XbaI, SpeI and Apal restrictive enzymes, respectively. Clonal relatedness was investigated through PFGE in a CHEF-DR III apparatus (Bio-Rad Laboratories, Athens, Greece). The running times were 23 h, with pulse times ranging from 3 to 20 s for *K. pneumoniae*; 24 h, with pulse times ranging from 10 to 30 s during the first 11 h and from 30 to 50 s during the next 13 h for *P. aeruginosa*; and 24 h, with pulse times from 5 to 30 s for *A. baumannii*, as previously described [21–23].
Table 1. Carbapenem-resistant *K. pneumoniae* strains – phenotypic, molecular and genotypic results

<table>
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<tr>
<th>Strain code</th>
<th>Isolation date (day/month/year)</th>
<th>Biological fluid</th>
<th>Carriage/infection</th>
<th>Genes detected</th>
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<th>PBA test†</th>
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<td>VIM</td>
<td>(+)</td>
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<td>B2</td>
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*Ethylenediaminetetraacetic acid test used for the phenotypic detection of metallo-beta-lactamase (MBL) production.
†Phenylboronic acid test used for the phenotypic detection of *K. pneumoniae* carbapenemase (KPC) production.

**Cluster analysis**

Cluster analysis of CR *K. pneumoniae, P. aeruginosa* and *A. baumannii* strains was performed using Bionumerics software version 7.1 (Applied Maths, Sint-Martens-Latem, Belgium).

Using the antimicrobial drug susceptibility plugin, the antimicrobial resistance data of CR strains were converted to SIR categories and dendrograms were calculated. The isolates were clustered based on their resistance using a categorical coefficient that treats different values as different states. Strains with 100 % similarity value were considered identical and grouped in the same phenotypic cluster.

Genotypic cluster analysis for all of the PFGE images was performed with Bionumerics using the unweighted-pair group method, with 1.5 % optimization values and 1.5 % position tolerance values for band comparison. Similarity coefficients were derived by calculating Dice coefficients. PFGE patterns with a similarity value >80 % [24], combined with the interpretation criteria proposed by Tenover et al. [25], were grouped in the same genotypic cluster.

**Diversity analysis**

Diversity analysis was performed using PAST software (version 3.11; University of Oslo, http://folk.uio.no/ohammer/past/). Analysis was carried out by calculating Simpson’s diversity index \([1-\Sigma(n_i/N)^2]\), where \(n_i\) is the total number of strains of a specific cluster and \(N\) is the total number of strains, and through computed rarefaction curves with 95 % CI. Simpson’s index of diversity is a measure of diversity that takes into account both richness and evenness. It represents the probability that two strains randomly and simultaneously selected from a sample belong to different clusters. Its value can range from 0, meaning that all isolates belong to the same phenotypic or genotypic cluster, 1, implying that each strain of the population belongs to a distinctive phenotypic or genotypic cluster. The number of clusters per sample is a measure of richness. Evenness is a measure of the relative abundance of different clusters. The more that richness and evenness increase, the more that diversity increases. Rarefaction is a tool to estimate cluster richness for a given number of sampled strains. Rarefaction curves are plots of the number of clusters,
i.e. phenotype or genotype, as a function of the number of strains sampled [26].

Simpson’s indices for bacterial phenotypic and genotypic clustering were compared using PAST software through the diversity permutation test. The level of statistical significance was set at $P$ value <0.05.

RESULTS
Phenotypic and molecular results
A total of 56 CR Gram-negative bacteria were studied. Among these, 28 (50 %) were K. pneumoniae isolates, 19 (33.9 %) were P. aeruginosa and 9 (16.1 %) were A. baumannii.

Among 28 CR K. pneumoniae strains, 16 (57.1 %) were KPC producers, 6 (21.4 %) were VIM producers, 3 (10.7 %) were OXA-48 producers, 2 (7.2 %) harboured both KPC and VIM genes, and 1 (3.6 %) carried no PCR-identified carbapenemase. All 16 KPC producers yielded a positive PBA test. All six VIM-producers yielded a positive EDTA test. Two out of the three OXA-48 producers yielded negative PBA and EDTA tests, while the isolate producing no PCR-identified carbapenemase PCR yielded negative phenotypic assays (Table 1).

Among the 19 CR P. aeruginosa strains, 17 (89.4 %) were VIM producers, 1 (5.3 %) was a KPC producer and 1 (5.3 %) produced no carbapenemase. Ten out of 17 (58.8 %) VIM producers yielded a positive EDTA test. The KPC producer and the strain with no carbapenemase yielded negative EDTA tests (Table 2).

All nine CR A. baumannii strains carried the intrinsic OXA-51 carbapenemase gene, while eight out of nine (88.9 %) carried the OXA-23 gene and one (11.1 %) transferred the OXA-58 carbapenemase gene. All nine strains yielded negative EDTA tests (Table 2).

Table 2. Carbapenem-resistant P. aeruginosa and A. baumannii strains – phenotypic, molecular and genotypic results

<table>
<thead>
<tr>
<th>Strain code</th>
<th>Strain type</th>
<th>Isolation date (day/month/year)</th>
<th>Biological fluid</th>
<th>Carriage/infection</th>
<th>Genes detected</th>
<th>EDTA test*</th>
<th>PFGE type</th>
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<td>OXA-51+OXA-23+TEM</td>
<td>(−)</td>
<td>A1</td>
</tr>
<tr>
<td>D1063</td>
<td>A. baumannii</td>
<td>17/05/12</td>
<td>Shunt</td>
<td>Carriage</td>
<td>OXA-51+OXA-23+TEM</td>
<td>(−)</td>
<td>A1</td>
</tr>
<tr>
<td>D1064</td>
<td>A. baumannii</td>
<td>23/08/12</td>
<td>Bronchial fluid</td>
<td>Carriage</td>
<td>OXA-51+OXA-23+TEM</td>
<td>(−)</td>
<td>A1</td>
</tr>
<tr>
<td>D1066</td>
<td>A. baumannii</td>
<td>03/12/12</td>
<td>Stool</td>
<td>Carriage</td>
<td>OXA-51+OXA-23+TEM</td>
<td>(−)</td>
<td>A1</td>
</tr>
<tr>
<td>D1182</td>
<td>A. baumannii</td>
<td>10/06/13</td>
<td>Trauma</td>
<td>Carriage</td>
<td>OXA-51+OXA-23+TEM</td>
<td>(−)</td>
<td>A1</td>
</tr>
<tr>
<td>D1065</td>
<td>A. baumannii</td>
<td>23/11/12</td>
<td>Blood</td>
<td>Infection</td>
<td>OXA-51+OXA-58+TEM</td>
<td>(−)</td>
<td>B</td>
</tr>
</tbody>
</table>

*Ethylendiaminetetraacetic acid test used for the phenotypic detection of metallo-beta-lactamase (MBL) production. †Integron-borne cephalosporinase. ‡Strains isolated 2 days before the beginning of the study period.
Phenotypic and genotypic clustering

According to their interpretative phenotypes, CR K. pneumoniae, P. aeruginosa and A. baumannii strains were grouped into 16 (A–P) (Table 1 and Fig. 1), 9 (Table 2 and Fig. 2) and 8 (A–H) (Table 2 and Fig. 3a) phenotypic clusters, respectively.

PFGE genotyping clustered the CR K. pneumoniae strains into seven clonal types (A–G). Clones A and B were each divided into two distinct subtypes (A1 and A2, and B1 and B2, respectively). All of the KPC producers belonged to clonal type A, while all of the VIM producers belonged to clonal type B. One strain that concurrently harboured blaVIM and blaKPC genes also belonged to clonal type B, while the second isolate, which concurrently transferred blaVIM and blaKPC genes, belonged to clonal type E. The strain that did not harbour a carbapenemase gene was classified as type C. The three OXA-48 producers belonged to three different clonal types (D, E and F) (Fig. 4).

PFGE analysis of CR P. aeruginosa strains generated profiles of six major clonal types (A–F). Clone A was divided into two subtypes (A1 and A2). VIM-producers were classified in to multiple clonal types (A, B, E and F). The KPC-producer belonged to a distinct pulsotype (C) and the strain not producing any carbapenemase belonged to a different pulsotype (D) (Fig. 5).

CR A. baumannii strains were clustered into two different clonal types (A and B) according to PFGE. Clone A was further divided into two subtypes (A1 and A2). All OXA-23 producers belonged to clone A, whereas the OXA-58-producing strain belonged to clone B (Fig. 3b).

Comparison of the phenotypic and genotypic diversity of CR Gram-negative bacterial strains

Using rarefaction curves to compare the phenotype with the genotype, we observed more phenotypic than genotypic clusters for a given number of strains for all three CR bacteria (Fig. 6). Likewise, the Simpson diversity index for the interpretative phenotype analysis and PFGE for CR K. pneumoniae strains was 0.89 and 0.6, respectively (P<0.001). For CR P. aeruginosa isolates the Simpson diversity index for the interpretative phenotype analysis and PFGE was 0.77 and 0.6, respectively (P=0.22). For CR A. baumannii strains, the Simpson diversity index for the phenotype and genotype was calculated as 0.86 and 0.19, respectively (P=0.004).
DISCUSSION

To the best of our knowledge, this is the first study to assess the genotypic and phenotypic diversity observed from PFGE and interpretative phenotype analysis of CR Gram-negative bacterial strains isolated from human clinical specimens. The results demonstrate that interpretative phenotype analysis and PFGE show different discriminatory power for CR *K. pneumoniae* and *A. baumannii* strains. They also show the first detection of a KPC-producing CR *P. aeruginosa* strain in Greece, as identified in other countries [27].

PFGE was selected as a genotypic typing method rather than multilocus sequence typing (MLST), as it is more suitable for single departments and local endemic areas. The KPC-producing CR *K. pneumoniae* strains emerging in our hospital display clonal dispersion [20], as in our case (Fig. 4), and usually belong to two different clonal types, with the ‘hyper-epidemic clone’ being predominant [28–30]. All of the VIM producers in our study belonged to clonal type B (Fig. 4), in accordance with the single-clone VIM epidemics previously recorded in our hospital [18, 31]. The cross-transmission of these strains between critically ill patients may be due to contaminated instruments or poor hand hygiene [32]. One CR *K. pneumoniae* strain that concurrently transferred *bla*<sub>VIM</sub> and *bla*<sub>KPC</sub> was also classified as clonal type B, as such strains are related to VIM producers [33].

The multiple OXA-48 pulsotypes and interpretative phenotypes revealed in our study (Figs 1 and 4) could be explained by the geographical proximity of our country to Turkey. In Greece, outbreaks of CR *K. pneumoniae* strains producing OXA-48-like carbapenemases are rare and usually only isolated cases have been detected [34, 35], but in Turkey multiple OXA-48-producing clones have been observed [36].

The significant difference observed between the genotypic and phenotypic diversity of CR *K. pneumoniae* strains arose because of the wide variety of interpretative phenotypic results for CS, TGC, amikacin (AMK), gentamicin (GEN) and co-trimoxazole (SXT) (Figs 1, 4 and 6), in accordance with local epidemiological data [30]. KPC-producing CR *K. pneumoniae* with different susceptibility profiles concerning these antimicrobial agents are classified as the MLST ST258 type, indicating genotypic relatedness to one other [37–40]. Moreover, they are not associated with new PFGE patterns [41]. Therefore, discriminating these with molecular epidemiological methods is generally difficult. The latter is in
accordance with our study, with respect to genotypic relat-
edness as revealed by PFGE. Providing CS and GEN are fre-
quently used for the treatment of CR *K. pneumoniae*
infections, resistance to these agents will keep on emerging,
producing variable antimicrobial profiles [30, 42].

The CR *P. aeruginosa* strains transferring *bla*$_{VIM}$ were clus-
tered in multiple pulsotypes (Fig. 5), as previously described for all of the VIM variants detected in our region to date [43–45]. This study constitutes the second report of *bla*$_{PER}$ ESBL isolation in a *P. aeruginosa* strain in Greece [23].

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**Fig. 3.** (a) Dendrogram of the nine carbapenem-resistant *A. baumannii* strains demonstrating their clustering and relatedness according to their interpretative phenotype. (b) Dendrogram of the nine PFGE-Apal identified carbapenem-resistant *A. baumannii* strains demonstrating their clustering and clonal relatedness.
The interpretative phenotype of CR *P. aeruginosa* strains shows higher diversity compared with genotypic fingerprinting, but not to a statistically significant level (Figs 2, 5 and 6). The fact that the majority of *P. aeruginosa* strains yielded VIM and were classified to multiple clones – in combination with the mechanisms of CR *P. aeruginosa* emergence, involving MBL acquisition, genetic events (e.g., mutations or insertions), efflux pump over-expression or porin inactivation [46] – meant that a large number of genotypes and interpretative phenotypes could be produced.

In our study, the phenotypic diversity of CR *P. aeruginosa* strains was due to various interpretative results for ceftazidime (CAZ), cefepime (FEP), aztreonam (ATM), piperacillin (PIP), piperacillin–tazobactam (TZP), AMK, GEN and CS (Fig. 2). Although MBLs normally hydrolyse CAZ and FEP, their mild hydrolytic activity is not always sufficient in itself to attain resistance to these agents, as in our case [44, 47, 48]. Only one VIM producer was susceptible to ATM, with all others exhibiting intermediate sensitivity, suggesting that other concomitant resistance mechanisms were present. The existence of other studies that present a broad spectrum of interpretative profiles for ATM among VIM producers strengthens this assumption [44, 45]. All VIM-producing *P. aeruginosa* strains presented intermediate sensitivity to PIP and TZP. Although this may seem unexpected, as VIM-producers normally display resistance to PIP and TZP, other observations record similar sensitivity to these agents [45]. As these agents have probably been used as therapeutic options against MBL-producing strains, the dominance of intermediately sensitive isolates may have occurred due to selective pressure.

The double-disk synergy test used for the phenotypic detection of MBL presented a relatively low sensitivity of 58.8 % for *P. aeruginosa* strains. Franklin *et al.* revealed higher sensitivities for this test in *K. pneumoniae* and *A. baumannii* strains [49]. However, in *P. aeruginosa* strains various sensitivities have been recorded for the assay, from 33.3 to 76.2 %, depending on the distance of the inhibitor and the substrates [50].

The majority of VIM-producing *P. aeruginosa* strains (94.1 %) showed resistance to AMK, while the interpretative patterns for GEN varied. VIM producers isolated in Greece have been concurrently carrying an aminoglycoside
resistance gene (aacA29a), which confers resistance to AMK but not to GEN [43, 44, 51], while for the variable GEN interpretative patterns, the involvement of additional aminoglycoside acetyltransferases has been proposed [45]. One CR *P. aeruginosa* isolate displayed intermediate sensitivity to CS. The emergence of CS resistance in these strains has been recorded in Greece [52].

Our study highlights the clonal spread of OXA-23-producing CR *A. baumannii* strains, as previously described in Greece (Fig. 3b) [53, 54]. Since 2010 there has been a massive replacement of the previously predominant OXA-58-producing strains by OXA-23 producers in Greece [53]. The higher MIC for MEM (≥16 mg l⁻¹) expressed by OXA-23 producers compared to that for the OXA-58 producer (8 mg l⁻¹) in our case probably provides them with a selective advantage to prevail [53]. All CR *A. baumannii* harboured the intrinsic *bla*OXA-51 carbapenemase gene, as expected [11, 55]. The activity of *bla*OXA-51 and *bla*OXA-23 against carbapenems and their ability to transfer from one strain to another was probably reinforced by the insertion sequence ISAba1, located upstream of these genes [56].

In accordance with our study, the number of CR *A. baumannii* clones detected at an international level is extremely limited, with these mostly belonging to the international clone II. This probably occurred because of the inability of typing technologies to resolve differences between closely related isolates, while the whole-genome sequence (WGS) provides detailed information on genetic differences among *A. baumannii* strains [57]. The significant difference observed between the genotypic and phenotypic diversity of CR *A. baumannii* strains crops up because of multiple interpretative phenotypic results for AMK, GEN, tobramycin, SXT, FEP and rifampin (RIF) (Figs 3a, b and 6). The CR *A. baumannii* strains isolated in our hospital displayed variable interpretative phenotypes regarding aminoglycosides [58]. The existence of the *aacA4* gene is strongly related to resistance to all aminoglycosides, except GEN, constituting the most frequently detected interpretative pattern in our study, while the emergence of the *aacC1* gene is associated with resistance to GEN [59]. One strain in our study unexpectedly displayed intermediate sensitivity to FEP, as the majority of MDR *A. baumannii* isolates in Greece show a high percentage of resistance to this antimicrobial agent [60].
Six out of nine (66.6 %) CR A. baumannii strains were phenotypically susceptible to SXT, although the majority of studies reveal higher resistance rates [61]. RIF has a significant synergistic effect against CR A. baumannii strains in combination with CS [62]. This probably triggers the emergence of resistance and the appearance of diverse interpretative phenotypes in respect of RIF. All CR A. baumannii strains were sensitive to CS, as CS resistance rates still remain low [62].

MDR bacterial infections can adversely affect all recipient outcomes from hospitalization, especially in cases of liver transplantation [3]. The horizontal spread of KPC- and VIM-producing CR K. pneumoniae and OXA-23-producing CR A. baumannii highlights the importance of strict infection control measures to reduce their spread in SOT departments [4]. In addition, the polyclonal OXA-48 CR K. pneumoniae strains and the emergence of CR isolates assigned to multiple clones, probably due to selective pressure, emphasize the need for the implementation of proper antimicrobial stewardship policies [5, 6].

Our study has the following limitations. (a) WGS, which is now considered to be the gold standard for genotypic analysis, was not used. However, this study covered a period between 2012 and 2013, and WGS was not available at our centres. Yang et al. compared WGS and PFGE and observed that neither method can definitively reveal the relatedness between CR K. pneumoniae isolates [63]. (b) CR bacteria were not screened for resistant genes regarding other antimicrobial agents. However, this study was conducted as part of a specific CR-screening project regarding colonization and/or infection in SOT patients. (c) CR bacteria were not tested for other mechanisms of carbapenem resistance that could affect gene expressiveness. Nevertheless, this was beyond the scope of this study.

**Conclusion**

KPC-producing K. pneumoniae strains and OXA-23 A. baumannii were clonally related, whereas VIM-producing P. aeruginosa strains had greater genetic diversity. CR K. pneumoniae and A. baumannii strains may have variability in their antimicrobial susceptibility profiles, despite being clonally related by PFGE. Thus, the presence of different antimicrobial susceptibility profiles does not preclude the possibility that two CR K. pneumoniae or A. baumannii isolates are clonally related.

**Funding information**

The authors received no specific grant from any funding agency.

**Acknowledgements**

We would like to thank Vassiliki Pentsioglou, Maria Karavade and Maria Malogianni for their valuable assistance during this study.

**Conflicts of interest**

The authors declare that there are no conflicts of interest.
Ethical statement
The study was carried out in accordance with the Declaration of Helsinki, as revised in 2008 and was approved by the Ethics Committee of Aristotle’s University Medical Faculty. Written informed consent by patient was not deemed by the Ethics Committee necessary for rectal swabs taken on a weekly basis.

References


