European Journal of Clinical Microbiology & Infectious Diseases (2019) 38:1547–1552
https://doi.org/10.1007/s10096-019-03585-0

ORIGINAL ARTICLE

Distribution of carbapenem resistance mechanisms in clinical isolates of XDR *Pseudomonas aeruginosa*

Annalisa De Rosa[1,2] · Nico T. Mutters[1,3] · Claudio M. Mastroianni[4] · Stefan J. Kaiser[1] · Frank Günther[1,5]

Received: 25 February 2019 / Revised: 7 May 2019 / Accepted: 9 May 2019 / Published online: 31 May 2019

Abstract

Our study aims to define the epidemiology of carbapenem resistance mechanisms in clinical isolates of *Pseudomonas aeruginosa* (PA). We evaluated 11,457 clinical PA strains isolated between 2009 and 2015 at the tertiary care University Hospital in Heidelberg, Germany. Thirty-four percent of the isolates (3867/11,457) were MDR (multidrug-resistant), 16% (1816/11,457) were XDR (extensively drug resistant), and less than 1% (82/11,457) had a PDR (pandrug-resistant) profile. Of those, 23% carried a carbapenemase gene (CPM positive) with 12% VIM-2, 10% VIM-1, and less than 1% IMP-1. Comparing MIC (minimal inhibitory concentration) distributions, the mean rank for meropenem, imipenem, gentamicin, and fosfomycin was significantly higher in the CPM-positive group than in the CPM-negative XDR group ($p \leq 0.004$). oprD (outer membrane protein) mutations were found in 19/19 tested strains; 12/19 carried a CPM and had a higher mutation rate. Meropenem resistance was mostly associated with the presence of CPM. Only 1/19 strains was meropenem resistant in the absence of CPM genes; nevertheless, it carried an oprD mutation in a strategic site (loop 2). Of 19 CPM-negative strains tested, 7 (36%) showed EP (efflux pumps) hyperexpression versus 12 in the CPM-positive strains. In our study, nearly 50% of the PA isolates exhibited resistance to the tested first-line antibiotics. Our study also demonstrates that carbapenemase genes can be isolated in approximately 23% of XDR PA strains in our population. This finding supports the clinical relevance of PA driven by the possible presence of multiple resistance mechanisms acquired under exposure to antibiotics or by horizontal transfer of resistance genes.

Keywords XDR · MDR · *Pseudomonas aeruginosa* · Carbapenemases · oprD · Efflux pumps

Abbreviations

PA	*Pseudomonas aeruginosa*
MDR	Multidrug-resistant
XDR	Extensively drug-resistant
PDR	Pandrug-resistant
PTZ	Piperacillin tazobactam
CAZ	Ceftazidime
GEN	Gentamicin
IMP	Imipenem
MEM	Meropenem
FOS	Fosfomycin
ICU	Intensive care unit
ESBL	Extended spectrum beta-lactamase
CPM	Carbapenemases
oprD	Outer membrane protein gene
OprD	Outer membrane protein amino acid sequence
EP	Efflux pumps
PaβN	Phe-Arg-β-naphthylamide
MBL	Metallo-beta-lactamases
MIC	Minimal inhibitory concentration

Annalisa De Rosa and Nico T. Mutters contributed equally to this work.

✉ Frank Günther
frank.guenther@staff.uni-marburg.de

[1] Centre for Infectious Diseases, Heidelberg University Hospital, Heidelberg, Germany

[2] Department of Internal Medicine III, Ulm University Hospital, Ulm, Germany

[3] Institute for Infection Prevention and Hospital Epidemiology, Medical Center – University of Freiburg, Freiburg, Germany

[4] Department of Infectious Diseases, Sapienza University of Rome, Rome, Italy

[5] Institute for Medical Microbiology and Hygiene, Marburg University Hospital, Marburg, Germany

Introduction

Pseudomonas aeruginosa (PA) is a common cause of serious healthcare-associated infections. Strains exhibiting multidrug resistance are responsible for an increasing number of infections, further hampering the efficacy of antibiotic treatment.

Of major concern is the emerging resistance to carbapenems, which has driven the WHO to include carbapenem-resistant PA on its list of pathogens of critical priority for the research and development of new antibiotics [1].

Carbapenem resistance arises from multiple mechanisms. PA intrinsically expresses efflux pumps (i.e., MexAB-OprM, MexCD-OprJ, and MexEF-OpnN/M) and increases their transcription levels in response to antibiotic pressure [2–4]. Moreover, dysfunction or lower expression of the outer membrane protein OprD plays an important role in carbapenem resistance as OprD is involved in carbapenem transport through the outer membrane into the periplasm [5–8].

However, enzyme production is the most important mechanism for resistance development and horizontal transmission of resistance. PA harbors a chromosomal beta-lactam inducible class C beta-lactamase (AmpC) with low-affinity to carbapenems; nevertheless, hyperproduction in combination with permeability alterations can easily lead to resistance. Notably, transferable plasmid-associated carbapenemase genes have been frequently found in PA. These mainly include class B or metallo-beta-lactamases (MBL), carbapenemases (i.e., VIM, IMP, SPM, and GIM) and, rarely, class D beta-lactamases such as OXA-40 [9, 10].

Multiple studies have investigated different resistance mechanisms in laboratory PA strains; our study has instead been conducted on a large collection of clinical isolates. Our investigations aim to define the local epidemiology of MDR (multidrug-resistant) and XDR (extensively drug-resistant) PA, to characterize carbapenemase gene distribution, the frequency and identification of oprD mutations, and the role of efflux pump hyperexpression in carbapenem resistance, and finally, to define the interplay of these mechanisms in MDR and XDR strains. We analyzed 11,457 clinical PA strains in total.

Materials and methods

Bacterial strains

Data from a total 11,457 clinical PA strains isolated between 2009 and 2015 from different departments of the Heidelberg University Hospital, a tertiary care center with more than 2000 beds, were collected. Clinical isolate was defined as bacterial strain obtained from in-hospital patients. The material the strains were isolated from are depicted in Table 1. A total of 965 clinical isolates classified as XDR according to criterion from Magiorakos et al. [11] were collected during the study period from different hospital departments. To exclude duplicates, 214 of the 965 XDR isolates, representing one distinct isolate per patient, were selected and used for further analysis. Data about isolation materials were retrospectively derived from the laboratory information system. The distribution across the different materials and departments is depicted in Tables 1 and 2.

Table 1 Distribution of *Pseudomonas aeruginosa* isolates by origin of isolation

Material	*n*	%
Urinary tract	2102	18.4
Skin	1370	12.0
Tracheal secretion	1141	10.0
Upper respiratory tract	574	5.0
Sputum	405	3.5
Blood	219	1.9
Bronchial secretion	195	1.7
Rectal swab	182	1.6
CVC	91	0.8
Other	5178	45.0
Total	11,457	100

Microbiological testing

Screening swab samples and diagnostic samples were inoculated on suitable media and incubated for 48 h at 36 °C. If growth on plates was detected, identification of microorganisms was performed by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (Bruker Daltonics, Bremen, Germany). Susceptibility testing was performed using VITEK2 (Biomérieux, Nuertingen, Germany), agar diffusion testing, or MIC test strips (Liofilchem, Piane Romano, Italy), as appropriate, and the results were interpreted according to the EUCAST clinical breakpoints v 6.0 [12]. For fosfomycin, interpretation was done using an epidemiological cutoff (ECOFF) value of ≤ 128 μg/ml. For selected strains, meropenem minimum inhibitory concentrations (MIC) were determined by a microdilution method according to the ISO 20776-1 recommendation [13].

Table 2 Distribution of *Pseudomonas aeruginosa* isolates by department

Department	*n*	%
Surgery	1942	17.0
Orthopedics	1823	15.9
Internal Medicine	1734	15.1
Pediatrics	1358	11.9
Urology	307	2.7
Gynecology	93	0.8
Others	4200	36.7
Total	11,457	100

Multiplex PCR technique

Bacterial DNA was extracted using TRIS-EDTA buffer containing glass beads (Carl Roth, Karlsruhe, Germany). The following PCR primers were designed to amplify internal fragments of 159 to 750 bp according to Kaiser et al. [14]. All strains were analyzed in two steps: first, strains were screened for carbapenemases using Multiplex PCR; and second, positive results were confirmed using a single PCR primer. Amplification was carried out using Taq DNA polymerase with Standard Taq Reaction Buffer (New England BioLabs, Frankfurt am Main, Germany) under the following conditions: 10 min 94 °C; 35 cycles: 45 s at 58 °C, 60 s at 72 °C, and 60 s at 94 °C; and 72 min at 72 °C for the final extension. The PCR products were analyzed by electrophoresis on a 1% agarose gel using GelRed™Nucleid Acid Gel Stain (Biotium, Dossenheim, Germany).

oprD sequencing

Sequencing of oprD was performed for selected isolates. DNA purification was performed using a DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) and PCR was performed using the primers oprD-F 5′-CGCCGACAAGAACT AGC-3′ and oprD-R 5′-GTCGATTACAGGATCGACAG-3′. Sequencing was completed by GATC Biotech Konstanz Germany. The deduced amino acid sequences were obtained using ExPASy (Expert Protein Analysis System) Translate tool (Swiss Institute of Bioinformatics). Alignment and sequence analyses were performed using MUSCLE (EMBL, Heidelberg, Germany) on the SeaView Multiplatform. *Pseudomonas aeruginosa* PAO1 from The Pseudomonas Genome Database was used as a reference strain [15].

Phenotypic analysis of Mex efflux pump overexpression

A phenotypic test to detect hyperexpression of efflux pumps was performed. We used a broad spectrum inhibitor for Mex pumps, Phe-Arg-β-naphthylamide (PaβN), at concentrations of 25 mg/L and 50 mg/L [5] to determine, in duplicate, the MICs for meropenem in the presence or absence of PaβN. Mex overexpression was assumed when the addition of the inhibitor induced a twofold reduction of MICs [5].

Statistics

A Wilcoxon signed-rank test was used to compare MIC distributions. p values ≤ 0.05 were regarded as statistically significant. Statistical analysis was performed using SPSS software 23.0 (SPSS, Chicago, IL, USA).

Results

Antimicrobial susceptibility testing

We analyzed the MIC values derived from 11,457 isolates for the following antibiotics: piperacillin tazobactam (PTZ), ceftazidime (CAZ), ciprofloxacin (CIP), gentamicin (GEN), imipenem (IMP), meropenem (MPM), and fosfomycin (FOS). Referring to ECDC recommendations, we defined strains resistant to 1 to 3 of the antibiotics PTZ, CAZ, CIP, GEN, IMP, or MEM as MDR; strains resistant to all of the above antibiotics as XDR; and strains resistant to PTZ, CAZ, CIP, GEN, IMP, and MEM as well as resistant to FOS as PDR. Approximately half (49%; 5682/11,457) of the isolates in our collection were susceptible to all the antibiotics described above, 34% (3867/11,457) were MDR, 16% (1816/11,457) were XDR, and less than 1% (82/11,457) exhibited a PDR profile.

Multiplex PCR technique

To exclude duplicates, 214 out of 965 XDR isolates, representing one distinct isolate per patient, were selected and used for further analysis. All chosen isolates were carbapenem resistant. Of this subset, 48/214 (23%) carried a carbapenemase gene (carbapenemase positive or CPM positive). The distribution of carbapenemases is depicted in Table 2.

We compared the MIC distribution in the XDR CPM-positive (48/214) versus the XDR CPM-negative (166/214) group. The mean ranks for meropenem, imipenem, gentamicin, and fosfomycin were significantly higher in the CPM-positive group than in the CPM-negative XDR group ($p \leq 0.004$).

The VIM-2-positive strains (26/214) had higher MICs for meropenem (mean rank 117.96 vs 98.70; $p = 0.46$), imipenem (mean rank 114 vs 99.24; $p = 0.052$), and gentamicin (mean rank 132.31 vs 94.38; $p = 0.001$) compared to all other XDR PA strains.

In the VIM-1-positive strains (20/214), there was no significant difference in the meropenem and imipenem MIC distributions compared to the CPM-negative strains. However, the VIM-1-positive isolates had higher MICs for gentamicin (mean rank 123.34 vs 97.54; $p = 0.045$) and fosfomycin (mean rank 69.50 vs 45.54; $p = 0.002$) compared to all other XDR PA strains.

Sequencing of the oprD gene

To explore the role of oprD mutations in determining carbapenem resistance in clinical isolates, we performed oprD sequencing on 19 strains randomly selected from our collection of 214 XDR. We classified the OprD amino acid sequences as one of the three different types.

Type I: short sequences of 16–137 aa (8 strains)
Type II: medium sequences of 220–276 aa (6 strains)

Type III: long sequences of 344–476 aa (5 strains)

Of the selected strains, 14/19 carried a carbapenemase gene (VIM-1, VIM-2, or IMP-1), and the OprD sequences from these isolates were randomly distributed among the 3 sequence types.

All the CPM-negative strains (5/19) had OprD sequence type I.

Type I sequences were characterized in 8 isolates.

Interestingly, a total of 6 strains (3 CPM positive and 3 CPM negative) had an OprD gene coding for a 137 aa sequence (type I) due to the premature stop codon TGG->TGA in position 412 [16, 17].

Of these strains, 4 had higher MICs to meropenem (MIC>16), and only one was CPM negative. When we compared this highly meropenem-resistant CPM-negative strain to the other CPM-negative strains (having a meropenem MIC of 8 μg/ml), we observed an additional OprD mutation (A614G) leading to the substitution of a serine (polar amino acid) with a glycin (apolar) (S121G) in loop 2, which is considered a carbapenem-binding site involved in meropenem and doripenem resistance. In the other 2 type I strains, oprD coded for a 16 aa and 80 aa sequence harboring a premature stop codon due to a frame shift and deletion mutation, respectively. Both isolates were CPM negative and had lower meropenem MICs (8 μg/ml) but differed in regard to their imipenem MICs (8 μg/ml and 16 μg/ml, respectively).

Type II sequences were characterized in 6 isolates; all were CPM positive with higher imipenem and meropenem MICs (MIC 16 μg/ml). The OprD sequences identified shared amino acid changes, namely, T103S, F170L, K115T, E185Q, P186G, and V189T (observed in IRMS (imipenem-resistant meropenem susceptible) strains) [18, 19]. Furthermore, in 4/6 strains, the oprD gene had the premature stop codon TGG->TAG in position 830, already described as one of the major mechanisms of OprD inactivation [16, 17].

Type III sequences were characterized in 5 CPM-positive strains, which all had high meropenem MICs (MIC 16 μg/ml). In 2/5 strains, OprD sequences had high similarity to the amino acid sequence coding the allele oprD TS [16].

Phenotypic analysis of MEX efflux pump overexpression

We randomly selected 22 XDR CPM-negative strains and performed a phenotypic analysis of MEX efflux pump overexpression. We performed a MIC microdilution test with meropenem in the presence or absence of PaβN. According to the literature, the presence of EP hyperexpression is demonstrated when the addition of the EP-inhibitor produces greater than a twofold reduction of the meropenem MIC [5]. In 12 strains, no EP hyperexpression was documented. Seven isolates showed hyperexpression of EP. No statistically significant difference in meropenem, gentamicin, or fosfomycin MICs was found between the two groups.

Discussion

The aim of our study was to characterize the epidemiology of *Pseudomonas aeruginosa* in our center, determining the prevalence of MDR, XDR, and PDR PA strains. Analyzing data from 11,457 isolates, we observed 34% MDR, 16% XDR, and less than 1% PDR.

Furthermore, we characterized the principal carbapenem resistance mechanisms and evaluated their distribution in the clinical setting.

In 214 XDR strains studied, 48 strains (23%) carried a carbapenemases gene. Our data align with the most recent report (epidemiological bulletin 2013) from the KRINKO commission of the Robert Koch Institute referring to German epidemiology of carbapenemase-producing Gram-negative *Pseudomonas aeruginosa* [20] in which they confirm that approximately 20% of carbapenem-resistant strains carry a carbapenemase gene. As the literature suggests, VIM-2 is the most frequent MBL in PA. Interestingly, in our samples, blaVIM-1 incidence is higher than expected, and this is probably related to the fact that the Heidelberg University clinic is a tertiary care center receiving international patients. For the same reason, we also studied other carbapenemase genes and found that GIM-1 and NDM-1 are worth further consideration. GIM-1, MBL German imipenemase, was identified for the first time in 2002 in extensively resistant clinical isolates (susceptible only to colistin and aztreonam); no positive samples were found in our data confirming this, as the blaGIM-1 gene seems to be confined to a very localized region [21–24].

NDM-1-producing PA was first isolated in 2011 in Serbia [25]; few NDM-positive strains have been reported in France, Italy, Slovakia, or Egypt and only 4 isolates have been characterized in India [26–28].

Our interest was in the epidemiology of NDM-1 PA, considering that there is an expected increase in NDM-1 incidence due to immigration from Asian countries. No NDM-1-producing PA was present in the XDR collected in our study, but we believe surveillance should be maintained in order to recognize possible outbreaks.

Our research demonstrates that in XDR strains, the presence of a carbapenemase gene is associated with increased carbapenem MICs. For instance, in a sub-analysis, VIM-2-positive strains had higher MICs for meropenem and imipenem; this observation could be explained by greater intrinsic enzyme activity, a different gene expression pattern in comparison to VIM-1 strains, or concurrence with other resistance mechanisms.

Moreover, CPM-positive strains, mainly VIM-1 carriers, showed increased MICs for gentamycin and fosfomycin. This finding may be due to the association between CPM

genes and other resistance genes on transposable genetic elements, as is the case with the 16S rRNA methylase gene responsible of aminoglycoside resistance, which was shown to be cotransported with carbapenemases [29–31].

We also demonstrated oprD mutations in all the XDR strains tested and confirmed that alteration of permeability is the most represented resistance mechanism in carbapenem nonsusceptible PA. Nevertheless, the interplay between carbapenemases and OprD mutations may have an impact on the level of meropenem resistance, mainly the type of mutation; however, the type of oprD mutation could be a driver for the increase in meropenem MICs. All the strains in which we studied oprD were IMP and MPM resistant; when looking at meropenem MICs, we observed that the presence of the CPM gene was always associated with a higher MIC, with the exception of 2 strains that had an oprD sequence identical to the allele TS identified in IRMS strains (imipenem resistant, meropenem susceptible) [32].

However, all the CPM-negative strains exhibited a lower meropenem MIC except one isolate in which we could detect an oprD substitution mutation (A614G) of a polar with a nonpolar amino acid in loop 2, which was identified as a meropenem-binding site [8].

Interestingly, in our collection, CPM-positive samples harbor a highly mutated oprD gene and may signify a longer period of evolution. Currently, oprD mutations are known to affect sensitivity to antibiotics other than carbapenems, and this could explain the higher MICs for gentamicin and fosfomycin in CPM-positive versus CPM-negative isolates.

Efflux pump hyperexpression was demonstrated in fewer than half of the CPM-negative strains and, in the selected strains, seemed to have no direct impact on MICs for carbapenems, aminoglycosides and fosfomycin. However, a small sample size may bias this aspect of the analysis. It is also probable that in this specific population with highly mutated oprD, efflux pump hyperexpression is not a major determinant in their resistance pattern.

Conclusions

XDR Pseudomonas aeruginosa represents a serious concern worldwide. In immunocompromised hosts, this bacterium can cause infections with a high rate of mortality. This mortality has been attributed to high antibiotic resistance and, consequently, to the delay of adequate therapy. Furthermore, there is the evidence that eradication of this pathogen from the clinical environment is very unlikely.

Our data clearly indicates that XDR strains producing CPM are more likely to induce therapeutic failure in our study population. Routine detection of carbapenemases in clinical PA isolates should be therefore used with regard to adequate therapy planning. On the other hand, rapid development of resistance by the diversity of inducible factors in PA is represented by the majority clinical isolates and should not be neglected as a problem. Further research is necessary to evaluate the phenotypic effect of concurrent resistance mechanisms in *Pseudomonas aeruginosa* and their impact on therapeutic decisions and infection control measures.

Funding The study was supported by a grant from the Ministry of Science, Research and the Arts of Baden-Württemberg (MWK) within the project "Surveillance von Mehrfach-Antibiotika-Resistenzen"; grant recipient NTM.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Ethical approval All used isolates were routinely collected in the microbiology laboratory of the Heidelberg University Hospital and stored at −70 °C. The current study thus is descriptive of those isolates. Data collected from patients was anonymized and restricted to possible clinical symptoms of infection. Ethical approval and informed consent statements were therefore not required.

References

1. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL et al (2018) Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 18(3):318–327
2. Webber MA, Piddock LJ (2003) The importance of efflux pumps in bacterial antibiotic resistance. J Antimicrob Chemother 51(1):9–11
3. Choudhury D, Das Talukdar A, Dutta Choudhury M, Maurya AP, Paul D, Dhar Chanda D et al (2015) Transcriptional analysis of MexAB-OprM efflux pumps system of Pseudomonas aeruginosa and its role in carbapenem resistance in a tertiary referral hospital in India. PLoS One 10(7):e0133842
4. Mesaros N, Glupczynski Y, Avrain L, Caceres NE, Tulkens PM, Van Bambeke F (2007) A combined phenotypic and genotypic method for the detection of Mex efflux pumps in Pseudomonas aeruginosa. J Antimicrob Chemother 59(3):378–386
5. Lomovskaya O, Warren MS, Lee A, Galazzo J, Fronko R, Lee M et al (2001) Identification and characterization of inhibitors of multidrug resistance efflux pumps in Pseudomonas aeruginosa: novel agents for combination therapy. Antimicrob Agents Chemother 45(1):105–116
6. Epp SF, Kohler T, Plesiat P, Michea-Hamzehpour M, Frey J, Pechere JC (2001) C-terminal region of Pseudomonas aeruginosa outer membrane porin OprD modulates susceptibility to meropenem. Antimicrob Agents Chemother 45(6):1780–1787
7. Ochs MM, Bains M, Hancock RE (2000) Role of putative loops 2 and 3 in imipenem passage through the specific porin OprD of Pseudomonas aeruginosa. Antimicrob Agents Chemother 44(7):1983–1985
8. Ochs MM, McCusker MP, Bains M, Hancock RE (1999) Negative regulation of the Pseudomonas aeruginosa outer membrane porin OprD selective for imipenem and basic amino acids. Antimicrob Agents Chemother 43(5):1085–1090
9. Poirel L, Naas T, Nordmann P (2010) Diversity, epidemiology, and genetics of class D beta-lactamases. Antimicrob Agents Chemother 54(1):24–38

10. Potron A, Poirel L, Nordmann P (2015) Emerging broad-spectrum resistance in Pseudomonas aeruginosa and Acinetobacter baumannii: mechanisms and epidemiology. Int J Antimicrob Agents 45(6):568–585
11. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG et al (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18(3):268–281
12. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 6.0, 2016. http://www.eucast.org
13. Standardization IO for. ISO 20776-1.2006 Clinical laboratory testing and in vitro diagnostic test systems — susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices — Part 1: reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases [Internet]. 2006. Report No.: 20776–1.2006. Available from: https://www.iso.org/obp/ui/#iso:std:iso:20776:-1:ed-1:v1:en
14. Kaiser SJ, Mutters NT, DeRosa A, Ewers C, Frank U, Günther F (2017) Determinants for persistence of Pseudomonas aeruginosa in hospitals: interplay between resistance, virulence and biofilm formation. Eur J Clin Microbiol Infect Dis 36(2):243–253
15. Winsor GL, Griffiths EJ, Lo R, Dhillon BK, Shay JA, Brinkman FSL (2016) Enhanced annotations and features for comparing thousands of Pseudomonas genomes in the Pseudomonas genome database. Nucleic Acids Res 44(Database issue):D646–D653
16. Gutierrez O, Juan C, Cercenado E, Navarro F, Bouza E, Coll P et al (2007) Molecular epidemiology and mechanisms of carbapenem resistance in Pseudomonas aeruginosa isolates from Spanish hospitals. Antimicrob Agents Chemother 51(12):4329–4335
17. El Amin N, Giske CG, Jalal S, Keijser B, Kronvall G, Wretlind B (2005) Carbapenem resistance mechanisms in Pseudomonas aeruginosa: alterations of porin OprD and efflux proteins do not fully explain resistance patterns observed in clinical isolates. APMIS. 113(3):187–196
18. Ocampo-Sosa AA, Cabot G, Rodriguez C, Roman E, Tubau F, Macia MD et al (2012) Alterations of OprD in carbapenem-intermediate and -susceptible strains of Pseudomonas aeruginosa isolated from patients with bacteremia in a Spanish multicenter study. Antimicrob Agents Chemother 56(4):1703–1713
19. Pragasam AK, Raghanivedha M, Anandan S, Veeraraghavan B (2016) Characterization of Pseudomonas aeruginosa with discrepant carbapenem susceptibility profile. Ann Clin Microbiol Antimicrob 15:12
20. KRINKO. Zur aktuellen Situation bei Carbapenemase-bildenden gramnegativen Bakterien Ein Bericht des NRZ für gramnegative Krankenhauserreger. RKI Robert Koch Institut
21. Wendel AF, Brodner AH, Wydra S, Ressina S, Henrich B, Pfeffer K et al (2013) Genetic characterization and emergence of the metallo-beta-lactamase GIM-1 in Pseudomonas spp. and Enterobacteriaceae during a long-term outbreak. Antimicrob Agents Chemother 57(10):5162–5165
22. Rieber H, Frontzek A, Pfeifer Y (2012) Emergence of metallo-beta-lactamase GIM-1 in a clinical isolate of Serratia marcescens. Antimicrob Agents Chemother 56(9):4945–4947
23. Kaase M, Szabados F, Pfennigwerth N, Anders A, Geis G, Pranada AB et al (2014) Description of the metallo-beta-lactamase GIM-1 in Acinetobacter pittii. J Antimicrob Chemother 69(1):81–84
24. Wendel AF, Kolbe-Busch S, Ressina S, Schulze-Robbecke R, Kindgen-Milles D, Lorenz C et al (2015) Detection and termination of an extended low-frequency hospital outbreak of GIM-1-producing Pseudomonas aeruginosa ST111 in Germany. Am J Infect Control 43(6):635–639
25. Jovcic B, Lepsanovic Z, Suljagic V, Rackov G, Begovic J, Topisirovic L et al (2011) Emergence of NDM-1 metallo-beta-lactamase in Pseudomonas aeruginosa clinical isolates from Serbia. Antimicrob Agents Chemother 55(8):3929–3931
26. Janvier F, Jeannot K, Tessé S, Robert-Nicoud M, Delacour H, Rapp C et al (2013) Molecular characterization of blaNDM-1 in a sequence type 235 Pseudomonas aeruginosa isolate from France. Antimicrob Agents Chemother 57(7):3408–3411
27. Rahman M, Shukla SK, Prasad KN, Ovejero CM, Pati BK, Tripathi A et al (2014) Prevalence and molecular characterisation of New Delhi metallo-β-lactamases NDM-1, NDM-5, NDM-6 and NDM-7 in multidrug-resistant Enterobacteriaceae from India. Int J Antimicrob Agents 44(1):30–37
28. team EC for DP and C (ECDC)-HCU-E editorial (2013) Isolation of NDM-1-producing *Pseudomonas aeruginosa* sequence type ST235 from a stem cell transplant patient in Italy, May 2013 [internet]. [cited 2017 Sep 20]. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20633
29. Yokoyama K, Arakawa Y (2003) Acquisition of 16S rRNA methylase gene in Pseudomonas aeruginosa. Lancet 362(9399):1888–1893
30. Fontes LC, Neves PR, Oliveira S, Silva KC, Hachich EM, Sato MIZ et al (2011) Isolation of Pseudomonas aeruginosa coproducing metallo-β-lactamase SPM-1 and 16S rRNA methylase RmtD1 in an Urban River▿. Antimicrob Agents Chemother 55(6):3063–3064
31. Gurung M, Moon DC, Tamang MD, Kim J, Lee YC, Seol SY et al (2010) Emergence of 16S rRNA methylase gene armA and cocarriage of blaIMP-1 in Pseudomonas aeruginosa isolates from South Korea. Diagn Microbiol Infect Dis 68(4):468–470
32. Edalucci E, Spinelli R, Dolzani L, Riccio ML, Dubois V, Tonin EA et al (2008) Acquisition of different carbapenem resistance mechanisms by an epidemic clonal lineage of Pseudomonas aeruginosa. Clin Microbiol Infect 14(1):88–90