DEVELOPMENT AND PILOT VALIDATION OF A NOVEL PCR-BASED REPLICON TYPING SCHEME FOR PLASMID FAMILIES ASSOCIATED WITH ANTIBIOTIC RESISTANCE IN *PSEUDOMONAS SPP*.

I. Stoikov[1,2], I. N. Ivanov[1], E. Dobreva[1], D. Donchev[1], S. Sabtcheva[2], R. Hristova[1]

[1] *National Reference Laboratory for Control and Monitoring of Antimicrobial Resistance, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria*
[2] *Laboratory for Clinical Microbiology, University specialized hospital for active treatment in oncology (National Oncology Center), Sofia, Bulgaria*

ABASTRACT

Background. *Pseudomonas* species are ubiquitous environmental Gram-negative bacteria increasingly associated with difficult to treat healthcare-associated infections. Along with their substantial intrinsic antimicrobial resistance, the ability to acquire additional resistance and pathogenicity determinants contributes to increased morbidity and mortality. Plasmids represent the major vehicles of gene transfer among hospital strains. Accumulation and dissemination of resistance genes through horizontal gene transfer is exceptionally problematic since it leads to the emergence of multi-resistant and stable phenotypes highlighting the importance of novel tools for studying plasmid epidemiology.

Materials and Methods. In this study we introduce a novel PCR-based replicon typing (PBRT) scheme for differentiation of various *Pseudomonas* spp. plasmid families requiring only two multiplex PCR (mPCR) assays. mPCR 1 is composed of previously published primer sets for IncP-1, IncP-7, IncP-9, IncQ, A/C, N, W, IncU. Primers for multiplex PCR 2 were designed after an in-depth *in-silico* bioinformatic analysis of the *repA* gene of more than 50 reference IncP-2, IncP-6, IncP-10, pKLC102-like and pMOS94-like plasmids some of which studied for the first time as a group.

Results. The scheme was tested on a set of 90 previously genotyped multi-resistant clinical *Pseudomonas* spp. isolates. The detection rate of the target plasmid families was low in our strain collection. Replicons were registered in only 3/90 isolates from the IncP-7 (n=1), IncP-10 (n=1), and pMOS94-like (n=1) families.

This pilot study demonstrates a novel PBRT scheme applicable to *Pseudomonas* spp. targeting plasmids of incompatibility groups known to harbour genes associated with antibiotic resistance.

Keywords:

Pseudomonas, PCR-based replicon typing (PBRT), plasmids, resistance

ADDRESS FOR CORRESPONDENCE:
Ivan N. Ivanov
National Reference Laboratory for Control and Monitoring of Antimicrobial Resistance,
National Center of Infectious and Parasitic Diseases,
26 Yanko Sakazov blvd 1504 Sofia, Bulgaria
Phone: +359 2 9446999 ext. 208
e-mail: ivanoov@gmail.com

INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative bacterium commonly associated with healthcare-associated infections. Intrinsic and acquired resistance mechanisms make infections caused by this opportunistic pathogen difficult to treat, resulting in increased morbidity and mortality. In *P. aeruginosa,* acquired resistance phenotypes are often associated with the acquisition of metallo-beta-lactamases (MBLs), of which VIM-type enzymes are most common, and extended-spectrum-beta-lactamases which are often co-transferred with aminoglycoside resistance determinants [1,2]. *bla*VIM and *bla*IMP MBL genes are usually located in mobile gene cassettes embedded in integron structures [3]. MBL-encoding integrons in *Enterobacterales* are mostly found on plasmids from IncN, IncI1 and IncHI2-incompatibility groups/types [4,5]. On the other hand, knowledge on beta-lactamase carrying plasmid lineages in *Pseudomonas* spp. is still scarce as only a limited number of plasmids have been completely sequenced and available through in genomic databases.

Plasmids are self-replicating extra-chromosomal circular fragments of DNA found in almost all

bacterial, but also in some fungal and plant species. They have the ability to acquire and lose genes and to be transferred horizontally between bacterial species by mobilization and conjugation. They are involved in the maintenance of bacterial genetic diversity and genomic plasticity. Plasmids carry genes for initiation and control of replication and may can contain genes encoding beneficial traits such as antimicrobial resistance and virulence factors [6]. They also possess systems that maintain their stability and copy number in the host cell.

The functions associated with initiation and control of plasmid replication are encoded by loci called replicons. The first PCR-based replicon typing (PBRT) scheme was by Carattoli proposed in 2005 and consists of multiplex PCRs targeting replicons of the major plasmid families among members of the *Enterobacterales* [6]. Initially, the scheme was able to detect 18 replicons belonging to 18 major incompatibility (Inc) groups (FIA, FIB, FIC, HI1, HI2, I1-Ig, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FIIA) [7]. Incompatibility between plasmids results from the dependence on the same replication machinery, i.e., two related plasmids cannot exist stably within the same cell line [4].

Another scheme for plasmid detection and classification is based on relaxase protein phylogenies, which target only transmissible plasmids. Conjugative plasmids carry the entire genetic machinery required for their transfer. These include origin of transfer (*oriT*), relaxase, coupling protein (T4CP) and type IV secretion system (T4SS). Mobilizable plasmids only encode only *oriT,* relaxase and the nicking accessory protein. They are not self-transmissible and their transfer is dependent on conjugative helper plasmids. Relaxases are the only common component of all transmissible plasmids. They are multidomain proteins with relaxase activity derived from their N-terminal domain. This scheme, called degenerate primer MOB typing (DPMT), utilizes the conserved N-terminal sequence of the relaxase proteins, on the basis of which plasmids are classified into six MOB families: MOB_F, MOB_P, MOB_Q, MOB_H, MOB_C and MOB_V [8].

The acquisition of additional resistance determinants by horizontal gene transfer is of particular importance to healthcare, as it leads to the emergence of highly resistant phenotypes that are difficult to treat [9], emphasizing the importance of methods to track the distribution of plasmids in the environment and in different hosts.

This study aims to introduce a novel PBRT scheme applicable to *Pseudomonas* spp. focusing on resistance plasmids as well as those potentially capable of carrying antimicrobial resistance genes.

MATERIALS AND METHODS

Detailed *in silico* analysis was performed on reference plasmid sequences downloaded from the NCBI GenBank (Table 1). Primer design was performed using Primer 3 software, implemented in Geneious Prime 2020.2.4 (https://www.geneious.com) and the pairs were additionally analyzed with Oligo7 [10]. The specificity of the designed primers was analyed using publicly available database (nr/nt) of NCBI through Primer-BLAST software (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). The diversity and comparison of nucleotide composition at replicon-containing regions was investigated using Geneious Prime 2020.2.4 and NCBI Blast. Finally, the scheme was tested on a set of 90 *P. aeruginosa* isolates selected from the collection of the National Reference Laboratory of Control and Monitoring of Antibiotic Resistance at the National Centre of Infectious and Parasitic Diseases in Sofia, Bulgaria. Bacterial DNA was extracted by boiling for 10 min in 10% Chelex 100 resin (Bio-rad) in TE buffer pH=8. Real-time multiplex touchdown PCRs were carried out in a final volume of 20µl with the following reagents: 1x PCR buffer (EURx) containing 2mM MgCl2 and 40 mM tetraethylammonium chloride, 0.35mM of each dNTP, 0.85x EvaGreen (Biotium), 0.03U/µl Perpetual Taq polymerase (EURx) and 1,5µl (~45ng) DNA. The optimal primer concentrations for multiplex PCR 1 and 2 were experimentally determined and are listed in Table 2. The thermal conditions were: 95°C initial denaturation for 3 min followed by 10 cycles of touchdown (with initial annealing temperature 10°C above primer Tm and decreasing by 1°C after each cycle), 20 cycles of 97°C for 10s, 55°C for 30s and 72°C for 60s (fluorescence detection at extension step), final extension at 72°C for 3 min followed by melting, starting with 10s at 72°C and then increasing by 0.4°C every 10s to 96°C. Positive PCR products were confirmed on a high-resolution QIAxcel capillary gel electrophoresis system (Qiagen, Hilden, Germany). Multiple alignments and phylogenetic trees were

Table 1. Reference plasmids and their replicon types used in the study

Plasmid	Replicon type	Accession no.
pCR1	IncP-10	NZ_CP020561
p14057-KPC	IncP-10	NZ_KY296095.1
p1011-KPC2	IncP-10	MH734334.1
R91-5 replication region DNA	IncP-10	X54695.1
p727-IMP	IncP-2	NZ_MF344568.1
RW109	IncP-2	NZ_LT969519.1
pSY153-MDR	IncP-2	NZ_KY883660.1
pJB37	IncP-2	KY494864.1
p12969-DIM	IncP-2	NZ_KU130294.1
pOZ176	IncP-2	NC_022344.1
pBM908	IncP-2	NZ_CP040126.1
pPABL048	IncP-2	CP039294.1
unnamed2	IncP-2	NZ_CP029096.1
unnamed3	IncP-2	NZ_CP029094.1
p1	IncP-2	CP027478.1
unnamed2	IncP-2	NZ_CP027170.1
pBM413	IncP-2	NZ_CP016215.1
pRBL16	IncP-2	NZ_CP015879.1
pTTS12	IncP-2	CP009975.1
pJBCL41	IncP-2	NZ_MK496050.1
pR31014-IMP	IncP-2	NZ_MF344571.1
pA681-IMP	IncP-2	NZ_MF344570.1
p12939-PER	IncP-2	NZ_MF344569.1
pCOL-1	IncP-6(IncG)	KC609323.1
pPA-2	IncP-6(IncG)	KC609322.1
pJB12	IncP-6(IncG)	KX889311.1
pD5170990	IncP-6(IncG)	NZ_KX169264.1
pMRVIM0713	IncP-6(IncG)	NZ_KP975076.1
p1	IncP-6(IncG)	NZ_CP040685.1
unnamed	IncP-6(IncG)	NZ_CP033834.1
pMATVIM-7	IncP-6(IncG)	NC_009739.1
Rms149	IncP-6(IncG)	NC_007100.1
p10265-KPC	IncP-6(IncG)	NZ_KU578314.1
pKLC102	pKLC102-like	AY257539.1
pROUE1	pKLC102-like	MK047608.1
pJB35	pKLC102-like	NZ_MF168945.1
pCOOP-101	pKLC102-like	NZ_MF141039.1
pAMBL1	pKLC102-like	NZ_KP873172.1
pNOR-2000	pKLC102-like	KC189475.1
Pseudomonas sp. MRSN12121 plasmid	pKLC102-like	CP010894.1
plasmid pAER57	pMOS94-like	NZ_MK671726.1
pMOS94	pMOS94-like	NZ_MK671725.1
pTROUS1	pMOS94-like	NZ_MK047610.1
pCB58	pMOS94-like	NZ_KY630469.1
pAMBL2	pMOS94-like	NZ_KP873171.1
pCT14	pMOS94-like	NC_010891.1
unnamed2	pMOS94-like	NZ_CP029092.1
unnamed1	pMOS94-like	NZ_CP027168.1
pIEC33019	pMOS94-like	NZ_CP016446.1
pPC9	pMOS94-like	NC_019906.1

Table 2. Primer concentrations in mPCR1 and mPCR2

Primer (F/R)	Concentration in 20 µl reaction (µM)	mPCR
IncP-1 (a,β,ε)	0,3	1
IncP-1γ	0,3	1
IncP-1γ-like	0,3	1
IncP-1δ	0,3	1
IncP-1ζ	0,3	1
IncP-7	0,35	1
IncP-9	0,4	1
IncQ	0,3	1
IncA/C (IncP-3)	0,2	1
IncN	0,25	1
IncW	0,2	1
IncU (IncG/IncP-6)	0,3	1
IncP-2	0,3	2
IncP-10	0,3	2
pKLC102-like	0,3	2
pMOS94-like	0,3	2

constructed after performing BLAST through the NCBI.

RESULTS AND DISCUSSION

Plasmids in *Pseudomonas* spp. were found to belong to at least 13 incompatibility groups (IncP-1 to IncP-7 and IncP-9 to IncP-14). IncP-2 plasmids tend to be large with a typical size of >300kb. Plasmids of IncP-2, IncP-5, IncP-7, IncP-10, IncP-12, and IncP-13 are characterized by a narrow host range. The rest, especially those belonging to IncP-1, IncP-4, and IncP-6 are described as broad-host range plasmids [11].

Analysis of primer characteristics and *in silico* defined product lengths led to the design of two multiplex PCRs. Previously published primer sets for IncP-1 (*trfA*) [12,13], IncP-7 (*rep*) [14], IncP-9 (*oriV-rep*) [15], IncQ (*oriV*) [16], IncA/C (*repA*), IncN (*repA*), IncW (*repA*) [6] and a newly designed primer pair for IncU (IncG/IncP-6) were grouped in **mPCR1 (Table 3).** Previously published primers for IncU targeting the *repA* gene within pRA3 plasmid from *Aeromonas hydrophila* [17] were aligned to the reference plasmids shown in Table 1. Comparison of primer binding sites and analysis with Primer BLAST showed that the primers were unable to cover most of the reference templates. Therefore, a new primer pair containing degenerate bases for IncU (*repA)* was designed and tested *in silico*. As a result, all the reference plasmid replicons were covered and the new primers were incorporated into mPCR1.

The IncP-1 incompatibility group corresponds to IncP in *E. coli*. Plasmids of this group have a wide host range and can be transferred and maintained in all Gram-negative bacteria [18]. They have a modular structure which that allows self-replication, stable maintenance, mating pair formation and DNA transfer. Most of these plasmids tend to carry at least one mobile genetic accessory element associated with antibiotic/metal resistance or xenobiotic degradation enzymes [19,20]. Based on the high diversity of the accessory elements, they are divided into subgroups: a, β, γ, δ, ε, ζ [12,21]. IncP-1 plasmids can confer resistance to carbenicillin, kanamycin, streptomycin, gentamicin, tobramycin, tetracyclines and sulfonamides [22]

IncP-9, IncP-7 and IncP-2 plasmids are involved in the catabolism of naphthalene and other aromatic hydrocarbons [23]. IncP-2 plasmids may be associated with resistance to carbenicillin, gentamicin, streptomycin, kanamycin, chloramphenicol, tetracycline and sulfonamides. IncP-7 plasmids have been reported to confer resistance to carbenicillin, gentamicin, streptomycin, tobramycin and sulfonamides. IncP-9 plasmids associated with resistance to carbenicillin, gentamicin, kanamycin, streptomycin, tobramycin, tetracycline and sulfonamide have also been reported in *Pseudomonas* spp. [22].

A plasmid of IncP-6 incompatibility group from *P. aeruginosa* was found to be capable of maintenance in *E. coli* where it was assigned to a new group called IncG. However, the two groups were considered to be the identical and were combined into the IncU incompatibility group [24]. IncU have broad-host range and have been reported to confer resistance to streptomycin, sulfanilamide, gentamicin, and carbenicillin [25]. A novel resistance plasmid (p10265-KPC) belonging to the IncP-6 incompatibility group was recently reported to harbor the bla_{KPC-2} gene, associated with resistance to almost all β-lactams [11].

IncQ plasmids have been reported to carry genes mainly associated with streptomycin and sulfonamide resistance. They are small, multicopy, nonconjugative and have a broad-host range [26].

Table 3. Primers used in PBRT multiplex PCR 1

Replicon type	Primers	Fragment Length (bp)	Reference
IncP-1 (a, β, ε)	Forward: TTCACSTTCTACGAGMTKTGCCAGGAC Reverse: GWCAGCTTGCGGTACTTCTCCC	281	[12]
IncP-1γ	Forward: TTCACTTTTTACGAGCTTTGCAGCGAC Reverse: GTCAGCTCGCGGTACTTCTCCCA	281	[12]
IncP-1γ-like	Forward: TTCACCTTCTACGAACTGTGTAAT Reverse: GTCAAGGCCCGATACTTCTCCCA	281	[13]
IncP-1δ	Forward: TTCACGTTCTACGAGCTTTGCACAGAC Reverse: GACAGCTCGCGGTACTTTTCCCA	281	[12]
IncP-1ζ	Forward: TTCACTTTCTACGAAATCTGCAAAGAC Reverse: GATAGCTTCCGATACTTTTCCCA	281	[13]
IncP-7	Forward: CCCTATCTCACGATGCTGTA Reverse: GCACAAACGGTCGTCAG	524	[14]
IncP-9	Forward: GAGGGTTTGGAGATCATWAGA Reverse: GGTCTGTATCCAGTTRTGCTT	610-637	[15]
IncQ	Forward: CTCCCGTACTAACTGTCACG Reverse: ATCGACCGAGACAGGCCCTGC	436	[16]
IncA/C (IncP-3)	Forward: GAGAACCAAAGACAAAGACCTGGA Reverse: ACGACAAACCTGAATTGCCTCCTT	465	[6]
IncN	Forward: GTCTAACGAGCTTACCGAAG Reverse: GTTTCAACTCTGCCAAGTTC	559	[6]
IncW	Forward: CCTAAGAACAACAAAGCCCCCG Reverse: GGTGCGCGGCATAGAACCGT	242	[6]
IncP-6 (IncU)	Forward: ACSGTGAAYASCAAGAGCGG Reverse: TGCTGCATCCGCTCGCCYTC	302	This study

IncA/C plasmids are often found in multi-drug resistant members of the *Enterobacterales*. They are self-mobilizing and have a broad host range. Their size usually varies between 131 and 195kb. Plasmids of this group often carry genes encoding ESBL, AmpC beta-lactamases and carbapenemases [27]. IncA/C plasmids from *E.coli* are equivalent to IncP-3 in *Pseudomonas* spp. [28].

IncN plasmids have been detected in clinical samples and various animal hosts. They are mostly associated with carbapenem and third generation cephalosporin resistance in *K. pneumoniae*, *K. oxytoca* and *E. coli* strains. In addition an IncN plasmid carrying bla_{NDM-1} was described in a multi-drug resistant *E. coli* isolate [29].

IncW incompatibility group consists of small, broad-host range plasmids with a wide spectrum of antibiotic resistance. Observations suggest that conjugation of these plasmids is only possible on a solid media [30]. One of the best-known members of this group is R388 with a size of 33kb. This plasmid is known to harbor genes encoding resistance to trimethoprim and sulfonamides [31].

For this study a total of 50 reference plasmid

Table 4. Primers used in multiplex PCR 2

Replicon type	Primers	Fragment Length (bp)	Reference
IncP-2	Forward: TCGTGAGGAGTTCGTTGAAG Reverse: TTCTGCGACTGAAGCTCTTT	126	This study
IncP-10	Forward: CAGCGAACAAATCATGGTGG Reverse: TGAGGTAGTCAAGCGTCAAC	325-332	This study
pKLC102-like	Forward: GACCCSGACAAGGATTTCAA Reverse: TTSGCSTKCGGATAGACG	74	This study
pMOS94-like	Forward: GTTGTGCTCGTAGSTGATCT Reverse: CGCAATCAACATCGACAAGT	304	This study

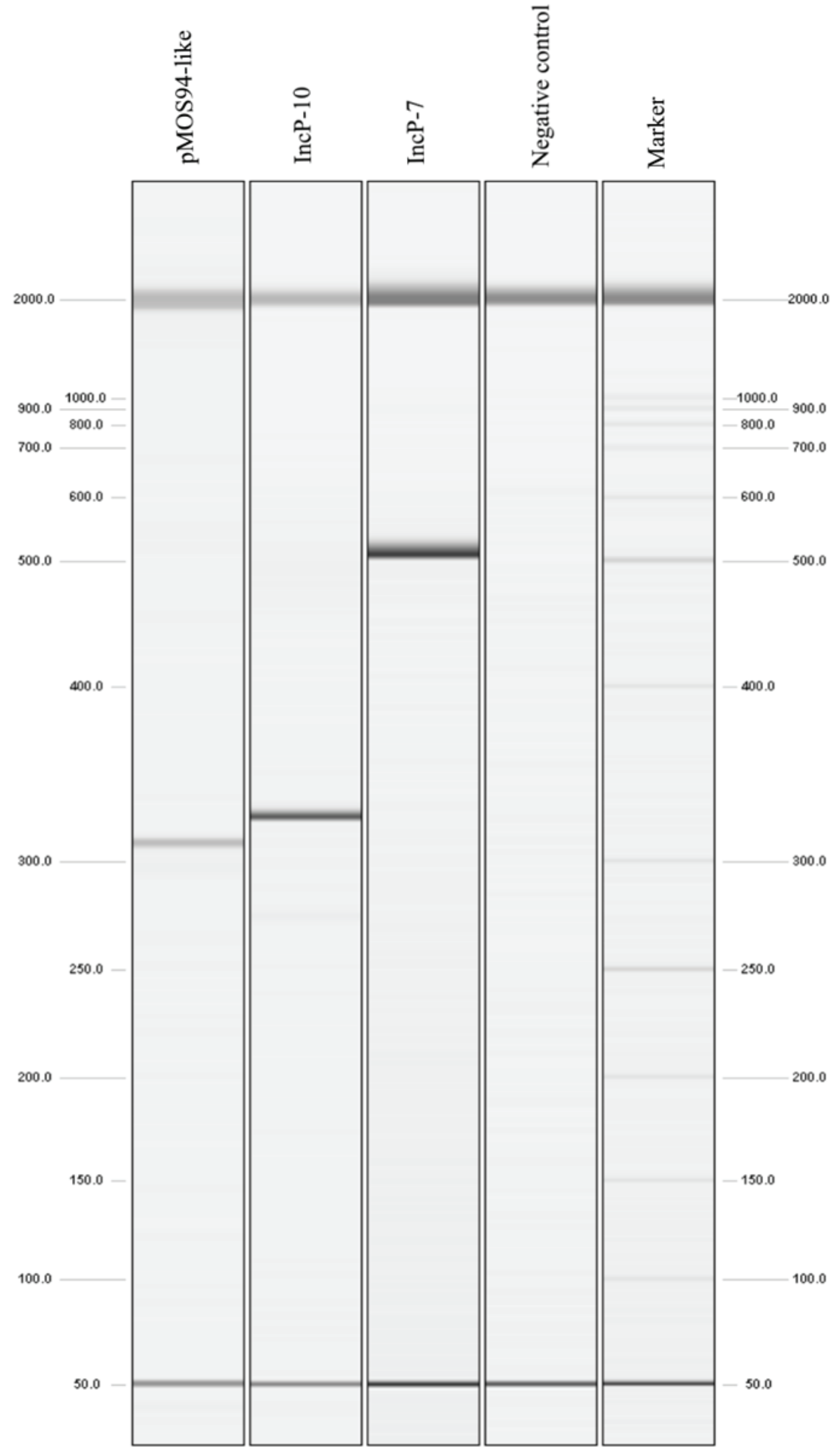

Figure 1. Representative capillary electrophoresis image of the replicons detected in this study

Figure 2. Phylogenetic tree (Neighbour-joining) generated by NCBI BLAST by querying a 304bp fragment from the *repA* gene of pMOS94 plasmid. A distinct cluster of pMOS94-like plasmids is shown forming a lineage.

sequences were downloaded from NCBI Genbank. Regions containing the replication initiation genes were extracted and analyzed by sequence alignment in *Geneious Prime 2020.2.4*. For several plasmid families primer design was performed directly on the aligned sequences taking into account and avoiding primer binding sites within regions of high diversity (Table 3 and 4).

Multiplex PCR 2 was designed with primers for IncP-2 (repA), IncP-10 (repA), pKLC102-like (repA) and pMOS94-like (repA) replicons (Table 4).

The plasmid pKLC102 is one of the few known examples of a mobile DNA coexisting simultaneously as a plasmid and as a genomic island in *P. aeruginosa* clone C strains [32]. Genomic islands are integrated into the genome at the 3' end of a tRNA gene. They are flanked by direct repeats and contain a transposase or integrase involved in the integration and excision processes. The genomic islands, also known as pathogenicity islands are clone- or strain-specific and are usually easily recognized by their atypical G+C content. They are related to the virulence of pathogenic strains by augmenting cell fitness, metabolic versatility and general adaptability [33].

A total of 90 *P. aeruginosa* isolates with known MLST and MLVA types were selected to test the scheme to achieve maximum heterogeneity. Amplification was observed in one isolate from multiplex PCR 1 and two isolates from multiplex PCR 2 Replicons were present in 3/90 (3,33%) isolates. Capillary electrophoresis analysis revealed three distinct products of 305bp, 327bp and 526bp, corresponding to pMOS94-like, IncP-10 and IncP-7, respectively (Fig. 1).

IncP-10 is known to confer resistance to carbenicillin, gentamicin, kanamycin, tobramycin and sulfonamides [22]. However, there are very few sequenced plasmids of this group and their properties are not fully understood.

Recently, pMOS94 was described as a *bla*$_{\text{VIM-1}}$-carrying plasmid [34]. The resolution and analysis of its structure led to the definition of a novel plasmid lineage associated with the spread of MBL genes among *Pseudomonas* spp. pMOS94 has been found to be untypable by the currently existing replicon typing schemes [34]. Our BLAST analysis of a 304bp fragment from the *repA* of the originally described pMOS94 (from *P. montelii*) showed that at least 11 additional plasmids could be classified into this lineage (**Figure 2**)

One of the limitations of this study was the lack of reference plasmid controls to confirm the *in silico* results *in vitro*. Therefore, the presence of plasmids and their corresponding replicons in the positive isolates will be further confirmed by S1-nuclease analysis and Southern blot hybridization which will also provide their approximate sizes. Based on the results from these analyses, plasmid DNA will be isolated and subjected to next-generation sequencing. In addition, the same subset of isolates is planned to be subjected to MOB typing in order to compare and evaluate the specificity and discriminatory power of the two assays.

CONCLUSIONS

Plasmids play an essential role in the dissemination of antibiotic resistance among bacterial communities. While this has become a serious threat for both human and animal healthcare, the need for reliable methods for detection, monitoring and control of antibiotic resistance is growing. The primary aim of this study was to develop a PBRT scheme which is able to detect plasmids of incompatibility groups known to harbor genes associated with antibiotic resistance dissemination among *Pseudomonas* spp. The scheme comprises of two multiplex PCRs including newly designed and previously published primer sets targeting known and recently reported replicons. The assay is affordable, easily implemented and could be used for tracking origins and routes of resistance plasmids across various settings, animal or hospital associated.

ACKNOWLEDGEMENT

The study was supported by the Bulgarian National Science Fund under Grant **КП-06-Н 23/5**

REFERENCES

1. Hong DJ, Bae IK, Jang IH, Jeong SH, Kang HK, Lee K. Epidemiology and characteristics of metallo-ß-lactamase-producing Pseudomonas aeruginosa. Infect Chemother 2015;47:81–97. https://doi.org/10.3947/ic.2015.47.2.81.
2. Vatcheva-Dobrevska R, Mulet X, Ivanov I, Zamorano L, Dobreva E, Velinov T, et al. Molecular epidemiology and multidrug resistance mechanisms of pseudomonas aeruginosa isolates from bulgarian hospitals. Microb Drug Resist 2013;19:355–61. https://doi.org/10.1089/mdr.2013.0004.
3. Partridge SR, Kwong SM, Firth N, Jensen SO. Mobile Genetic Elements Associated with Antimicrobial Resistance. Clin Microbiol Rev 2018;31:e00088-17. https://doi.org/10.1128/CMR.00088-17.

4. Carattoli A. MINIREVIEW Resistance Plasmid Families in Enterobacteriaceae. Antimicrob Agents Chemother 2009;53:2227–38. https://doi.org/10.1128/AAC.01707-08.
5. Villa J, Viedma E, Brañas P, Orellana MA, Otero JR, Chaves F. Multiclonal spread of VIM-1-producing Enterobacter cloacae isolates associated with In624 and In488 integrons located in an IncHI2 plasmid. Int J Antimicrob Agents 2014;43:451–5. https://doi.org/10.1016/j.ijantimicag.2014.02.006.
6. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 2005;63:219–28. https://doi.org/10.1016/j.mimet.2005.03.018.
7. Couturier M, Bex F, Bergquist PL, Maas WK. Identification and classification of bacterial plasmids. Microbiol Rev 1988;52:375–95. https://doi.org/10.1128/mmbr.52.3.375-395.1988.
8. Alvarado A, Garcillán-Barcia MP, de la Cruz F. A degenerate primer MOB typing (DPMT) method to classify gamma-proteobacterial plasmids in clinical and environmental settings. PLoS One 2012;7:40438. https://doi.org/10.1371/journal.pone.0040438.
9. Hawkey PM, Warren RE, Livermore DM, McNulty CAM, Enoch DA, Otter JA, et al. Treatment of infections caused by multidrug-resistant gram-negative bacteria: Report of the British society for antimicrobial chemotherapy/healthcare infection society/british infection association joint working party. J Antimicrob Chemother 2018;73:iii2–78. https://doi.org/10.1093/jac/dky027.
10. Rychlik W. OLIGO 7 primer analysis software. Methods Mol Biol 2007;402:35–59. https://doi.org/10.1007/978-1-59745-528-2_2.
11. Dai X, Zhou D, Xiong W, Feng J, Luo W, Luo G, et al. The IncP-6 plasmid p10265-KPC from Pseudomonas aeruginosa carries a novel ΔISEc33-associated blaKPC-2 gene cluster. Front Microbiol 2016;7:310. https://doi.org/10.3389/fmicb.2016.00310.
12. Bahl MI, Burmølle M, Meisner A, Hansen LH, Sørensen SJ. All IncP-1 plasmid subgroups, including the novel ε subgroup, are prevalent in the influent of a Danish wastewater treatment plant. Plasmid 2009;62:134–9. https://doi.org/10.1016/j.plasmid.2009.05.004.
13. Wolters B, Kyselková M, Krögerrecklenfort E, Kreuzig R, Smalla K. Transferable antibiotic resistance plasmids from biogas plant digestates often belong to the IncP-1ε subgroup. Front Microbiol 2015;5:765. https://doi.org/10.3389/fmicb.2014.00765.
14. Izmalkova TY, Sazonova OI, Sokolov SL, Kosheleva IA, Boronin AM. The P-7 incompatibility group plasmids responsible for biodegradation of naphthalene and salicylate in fluorescent pseudomonads. Microbiology 2005;74:290–5. https://doi.org/10.1007/s11021-005-0065-0.
15. Dealtry S, Ding GC, Weichelt V, Dunon V, Schlüter A, Martini MC, et al. Cultivation-independent screening revealed hot spots of IncP-1, IncP-7 and IncP-9 plasmid occurrence in different environmental habitats. PLoS One 2014;9. https://doi.org/10.1371/journal.pone.0089922.
16. Götz A, Pukall R, Smit E, Tietze E, Prager R, Tschäpe H, et al. Detection and characterization of broad-host-range plasmids in environmental bacteria by PCR. Appl Environ Microbiol 1996;62:2621–8. https://doi.org/10.1128/aem.62.7.2621-2628.1996.
17. García-Fernández A, Fortini D, Veldman K, Mevius D, Carattoli A. Characterization of plasmids harbouring qnrS1, qnrB2 and qnrB19 genes in Salmonella. J Antimicrob Chemother 2009;63:274–81. https://doi.org/10.1093/jac/dkn470.
18. Popowska M, Krawczyk-Balska A. Broad-host-range IncP-1 plasmids and their resistance potential. Front Microbiol 2013;4:44. https://doi.org/10.3389/fmicb.2013.00044.
19. Dennis JJ. The evolution of IncP catabolic plasmids. Curr Opin Biotechnol 2005;16:291–8. https://doi.org/10.1016/j.copbio.2005.04.002.
20. Schlüter A, Szczepanowski R, Pühler A, Top EM. Genomics of IncP-1 antibiotic resistance plasmids isolated from wastewater treatment plants provides evidence for a widely accessible drug resistance gene pool. FEMS Microbiol Rev 2007;31:449–77. https://doi.org/10.1111/j.1574-6976.2007.00074.x.
21. Norberg P, Bergström M, Jethava V, Dubhashi D, Hermansson M. The IncP-1 plasmid backbone adapts to different host bacterial species and evolves through homologous recombination. Nat Commun 2011;2:268. https://doi.org/10.1038/ncomms1267.
22. The Biology of Pseudomonas. Elsevier; 1986. https://doi.org/10.1016/b978-0-12-307210-8.x5001-5.
23. Izmalkova TY, Mavrodi D V, Sokolov SL, Kosheleva IA, Smalla K, Thomas CM, et al. Molecular classification of IncP-9 naphthalene degradation plasmids. Plasmid 2006;56:1–10. https://doi.org/10.1016/j.plasmid.2005.12.004.
24. Haines AS, Cheung M, Thomas CM. Evidence that IncG (IncP-6) and IncU plasmids form a single incompatibility group. Plasmid 2006;55:210–5. https://doi.org/10.1016/j.plasmid.2005.11.003.
25. Haines AS, Jones K, Cheung M, Thomas CM. The IncP-6 plasmid Rms149 consists of a small mobilizable backbone with multiple large insertions. J Bacteriol 2005;187:4728–38. https://doi.org/10.1128/JB.187.14.4728-4738.2005.
26. Rawlings DE, Tietze E. Comparative Biology of IncQ and IncQ-Like Plasmids. Microbiol Mol Biol Rev 2001;65:481–96. https://doi.org/10.1128/mmbr.65.4.481-496.2001.
27. Hazen TH, Zhao L, Boutin MA, Stancil A, Robinson G, Harris AD, et al. Comparative genomics of an IncA/C multidrug resistance plasmid from Escherichia coli and Klebsiella isolates from intensive care unit patients and the utility of whole-genome sequencing in health care settings. Antimicrob Agents Chemother 2014;58:4814–25. https://doi.org/10.1128/AAC.02573-14.
28. Yano H, Shintani M, Tomita M, Suzuki H, Oshima T. Reconsidering plasmid maintenance factors for computational plasmid design. Comput Struct Biotechnol J 2019;17:70–81. https://doi.org/10.1016/j.csbj.2018.12.001.
29. Poirel L, Bonnin RA, Nordmann P. Analysis of the resistome of a multidrug-resistant NDM-1-producing Escherichia coli strain by high-throughput genome sequencing. Antimicrob Agents Chemother 2011;55:4224–9. https://doi.org/10.1128/AAC.00165-11.
30. Fernández-López R, Garcillán-Barcia MP, Revilla C, Lázaro M, Vielva L, de la Cruz F. Dynamics of the IncW genetic backbone imply general trends in conjugative plasmid evolution. FEMS Microbiol Rev 2006;30:942–66. https://doi.org/10.1111/j.1574-6976.2006.00042.x.
31. Bolland S, Llosa M, Avila P, De F, Cruz LA. General Organization of the Conjugal Transfer Genes of the IncW Plasmid R388 and Interactions between R388 and IncN and IncP Plasmids Downloaded from. vol. 172. 1990.
32. Klockgether J, Reva O, Larbig K, Tümmler B. Sequence Analysis of the Mobile Genome Island pKLC102 of Pseudomonas aeruginosa C. J Bacteriol 2004;186:518–34. https://doi.org/10.1128/JB.186.2.518-534.2004.
33. Klockgether J, Würdemann D, Reva O, Wiehlmann L, Tümmler B. Diversity of the abundant pKLC102/PAGI-2 family of genomic islands in Pseudomonas aeruginosa. J Bacteriol 2007;189:2443–59. https://doi.org/10.1128/JB.01688-06.
34. Di Pilato V, Antonelli A, Giani T, Henrici De Angelis L, Rossolini GM, Pollini S. Identification of a Novel Plasmid Lineage Associated With the Dissemination of Metallo-β-Lactamase Genes Among Pseudomonads. Front Microbiol 2019;10:1504. https://doi.org/10.3389/fmicb.2019.01504.