

Article

Genomic Characterization of Carbapenemases in *Providencia* Species from Croatia: The Results of the Multicenter Study

Jasmina Vraneš ^{1,2,*}, Branka Bedenić ^{1,3}, Gernot Zarfel ⁴, Josefa Luxner ⁴, Andrea Grisold ⁴, Rocio Arazo del Pino ⁵, Tessa Burgwinkel ⁵, Haris Car ⁶, Maja Anušić ², Vladimira Tičić ², Marina Bubonja-Šonje ⁷, Sanda Sardelić ⁸ and Paul G. Higgins ^{5,9}

- ¹ Department of Medical Microbiology and Parasitology, University of Zagreb School of Medicine, 10000 Zagreb, Croatia; branka.bedenic@kbc-zagreb.hr
 - ² Department of Clinical Microbiology, Dr Andrija Štampar Teaching Institute of Public Health Zagreb, 10000 Zagreb, Croatia; maja.anusic@stampar.hr (M.A.); vladimira.ticic@stampar.hr (V.T.)
 - ³ Clinical Department for Clinical Microbiology, Infection Control and Prevention, University Hospital Center Zagreb, University of Zagreb, 10000 Zagreb, Croatia
 - ⁴ Diagnostic and Research Institute for Hygiene, Microbiology and Environmental Medicine, Medical University Graz, A-8010 Graz, Austria; gernot.zarfel@medunigraz.at (G.Z.); andrea.grisold@medunigraz.at (A.G.)
 - ⁵ Institute for Medical Microbiology, Immunology and Hygiene, Faculty of Medicine and University Hospital Cologne, University of Cologne, 50925 Cologne, Germany; rocio.arazo-del-pino@uk-koeln.de (R.A.d.P.); tessa.burgwinkel@uk-koeln.de (T.B.); paul.higgins@uni-koeln.de (P.G.H.)
 - ⁶ Secondary Health School Zagreb, 10000 Zagreb, Croatia; carharis112@gmail.com
 - ⁷ Department of Microbiology and Parasitology, Faculty of Medicine, University of Rijeka, 51000 Rijeka, Croatia; marina.bubonja@uniri.hr
 - ⁸ Department of Microbiology and Parasitology, University Hospital of Split, 21000 Split, Croatia
 - ⁹ Partner Site Bonn-Cologne, German Center for Infection Research (DZIF), 50931 Cologne, Germany
- * Correspondence: jasmina.vranes@stampar.hr; Tel.: +385-95-4678-000

Abstract

Background/objectives: A rise in infections associated with carbapenem-resistant *Providencia* species (CRPS) has been observed worldwide. This study presents a genomic analysis of CRPS isolates from four hospitals in Croatia and the outpatient setting, in order to determine the extent of the spread of CRPS in Croatia. In the present study, we applied a combination of phenotypic characterization and molecular analysis of resistance traits to determine the mechanisms and the routes of spread of CRPS. **Material and methods:** The antibiotic susceptibility testing was performed using disk-diffusion and broth dilution methods. The nature of extended-spectrum β -lactamases (ESBLs), carbapenemases, and fluoroquinolone resistance determinants was investigated by polymerase chain reaction (PCR). In order to obtain an insight into the whole resistome, selected isolates were subjected to the Interarray Genotyping Kit CarbaResist and whole genome sequencing (WGS). **Results:** In total, 30 isolates were collected from four centers, located in different geographic regions of Croatia. There was uniform resistance to piperacillin-tazobactam, cefuroxime, expanded-spectrum cephalosporins (ESCs), imipenem, ertapenem, meropenem, and ciprofloxacin. Immunochromatographic testing and PCR revealed OXA-48 and NDM carbapenemase in 15 isolates, respectively. Phenotypic tests for ESBLs were positive in all OXA-48 and one NDM-positive organism (16 isolates). The isolates were categorized as extensively drug-resistant (XDR). OXA-48-producing isolates were susceptible only to ceftazidime-avibactam, whereas NDM producers were susceptible to ceftiderocol and, in the majority of cases, also to amikacin. WGS identified a plethora of genes encoding resistance to aminoglycosides, such as *aadA1* and *aadA2*, *(aph(3'')-Ib)* and *(aph(6)-Id)*, sulfonamides *sul1* and *sul2*, trimethoprim *dfrA1*, *dfrA10*, and *dfrA12*, tetracyclines *tet(A)* and *tet(B)*, and chloramphenicol *catA3* and



Academic Editor: Michael Calcutt

Received: 30 October 2025

Revised: 14 January 2026

Accepted: 1 February 2026

Published: 8 February 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the

Creative Commons Attribution (CC BY) license.

catA5. Conclusions: *Providencia* spp., in spite of being a rare pathogen, should be included in the surveillance studies across the medical centers in Croatia.

Keywords: *Providencia stuartii*; carbapenemase; OXA-48; NDM; resistance

1. Introduction

Providencia spp. belong to the genus *Morganellaceae* within the family *Enterobacterales* and are increasingly identified as causative agents of urinary tract infections (UTI), wound infections, and skin and soft-tissue infections. Recently, a rise in infections caused by carbapenem-resistant *Providencia* species (CRPS) has been observed worldwide [1].

The species most frequently involved in human infections are *Providencia stuartii* and *Providencia rettgeri* [1]. They have intrinsic resistance to natural and semisynthetic penicilins, β -lactam-inhibitor combinations, and first-generation cephalosporins, due to the production of AmpC Ambler class C β -lactamase. Overproduction of AmpC can be due to induction or derepression and is associated with resistance to second- and third-generation or extended-spectrum cephalosporins (ESCs) such as ceftazidime, cefotaxime, and ceftriaxone. Resistance to ESC can also be mediated by production of extended-spectrum β -lactamases (ESBL) or overproduction of chromosomal or acquisition of plasmid-mediated AmpC β -lactamases (p-AmpC) [2].

The three main families of ESBLs are: TEM (Temoniera), SHV (suphydryle-variable), and CTX-M (cefotaximase-Munich). TEM and SHV variants are derived by mutations of parental broad-spectrum TEM-1, TEM-2, and SHV-1 β -lactamases [2]. CTX-M β -lactamases are class A enzymes, which are not closely related to TEM or SHV β -lactamases [3,4]. In contrast to TEM and SHV β -lactamases, which rely on amino acid substitutions to extend their substrate profile, CTX-M enzymes are native ESBLs. CTX-M β -lactamases are divided into five phylogenetic clusters based on their protein sequences: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25. AmpC β -lactamases hydrolyze ESC, monobactams, and cephamycins but spare fourth-generation cephalosporins and carbapenems. Unlike most ESBLs, they are not susceptible to inhibition with clavulanic acid, sulbactam, or tazobactam [5]. Carbapenems are the antibiotics of choice for treating infections caused by derepressed Amp-C or ESBL producers. However, increased use of carbapenems has led to the emergence of carbapenemases in *Enterobacterales*, including *Providencia* spp. The widespread occurrence of ESBL-producing *Providencia* spp. was noticed earlier, but the dissemination of CRPS has been observed much later [1].

Resistance to carbapenems in *Providencia* spp. is mediated primarily by two main mechanisms. The first involves overexpression of β -lactamase, such as AmpC or an ESBL, combined with decreased permeability due to porin loss or alteration. The second mechanism is related to the production of carbapenem-hydrolyzing β -lactamases belonging to Ambler class A serine β -lactamases (KPC, GES), class B metallo- β -lactamases (MBL) of the IMP, VIM, or NDM family, and class D OXA-48-like β -lactamases, also known as carbapenem-hydrolyzing oxacillinases (CHDL) [6,7]. Production of carbapenemases in *Providencia* species poses a serious therapeutic problem because of their intrinsic resistance to polymyxins and tigecycline [1].

Croatia belongs to the Southeastern European countries with a high rate of carbapenem-resistant *Enterobacterales* (CRE). While carbapenemases in other *Enterobacterales* species have been extensively investigated before, there are no bibliographical data referring to CRPS. This study presents a genomic analysis of CRPS isolates from four hospitals in Croatia and outpatients setting, in order to determine the extent of spread of CRPS in Croatia. In the

present study, we applied a combination of phenotypic characterization and molecular analysis of resistance traits in order to determine the mechanisms and routes of spread of CRPS. All isolates were first screened by phenotypic methods and conventional PCR to determine resistance phenotype and presence of β -lactam and fluoroquinolone resistance genes. Selected isolates were then analyzed using new molecular techniques to determine the whole resistome. The second goal was to compare resistance patterns among isolates with different carbapenemase types.

2. Materials and Methods

2.1. Bacterial Isolates

The isolates exhibiting reduced susceptibility to at least one carbapenem (imipenem, meropenem, or ertapenem) licensed in Croatia were collected from Dr Andrija Štampar Teaching Public Health Institute (ASPH) covering the outpatient setting in Zagreb and Sv. Ivan Zelina psychiatric hospital in Zagreb, University Hospital Center Sestre Milosrdnice (UHCSM) in Zagreb, University Hospital of Split (UHS), and Clinical Hospital Center Rijeka (CHCR) located in the coastal region of Croatia, from 19 March 2023 to 28 June 2025. The isolates were sent to the Clinical Department for Clinical Microbiology, Infection Prevention and Control of the University Hospital Centre Zagreb for phenotypic and molecular analysis. The taxonomy was investigated using MALDI-TOF MS (matrix-assisted laser desorption ionization–time of flight mass spectrometry) (Vitek MS, Bruker, Bremen, Germany). The selected isolates were subjected to the whole resistome analysis by an Interarray CarbaResist Kit, Bad Langensalza, Germany at the Diagnostic and Research Institute for Hygiene, Microbiology, and Environmental Medicine of the University in Graz, Austria, and to the Institute for Medical Microbiology, Immunology, and Hygiene of the University in Cologne, Germany, for whole genome sequencing (WGS).

2.2. Antimicrobial Susceptibility Testing and Phenotypic Tests for Detection of ESBLs, AmpC β -Lactamases, and Carbapenemases

The antimicrobial susceptibility profiles were verified by a disk-diffusion test (DDT) according to EUCAST guidelines [8] and the broth dilution method, following the latest version of the Clinical Laboratory Standard Institution (CLSI) guidelines [9]. The panel for routine DDT contained: amoxicillin (AMX 25 μ g), amoxicillin-clavulanate (AMC-20/10 μ g), piperacillin-tazobactam (TZP-110 μ g), cephalexin (CN-30 μ g), cefuroxime (CXM-30 μ g), ceftazidime (CAZ-10 μ g), cefotaxime (CTX-10 μ g), ceftriaxone (CRO-10 μ g), cefepime (FEP-30 μ g) imipenem (IMI-10 μ g), meropenem (MEM-10 μ g), ertapenem (ERT-10 μ g), gentamicin (GM-10 μ g), amikacin (AMI-30 μ g), norfloxacin (NOR), ciprofloxacin (CIP-5 μ g), levofloxacin (LVX-5 μ g), chloramphenicol (CHL-30 μ g), ceftazidime (FOX-30 μ g), ceftazidime-avibactam (CZA-14 μ g), and cefiderocol (FDC-30 μ g) and, for urine samples, additionally, sulphamethoxazole-trimethoprim (SMX-25 μ g) (Oxoid, Basingstoke, UK). The minimum-inhibitory concentrations (MICs) were determined by the broth microdilution method in Mueller–Hinton broth (MHB) (Biolife Italiana, Milan, Italy) to assess susceptibility to 13 antibiotics: amoxicillin alone and in combination with clavulanic acid, piperacillin-tazobactam, cefuroxime, ceftazidime, cefotaxime, ceftriaxone, cefepime, imipenem, meropenem, gentamicin, amikacin, and ciprofloxacin according to CLSI [9] except for amoxicillin alone, for which EUCAST criteria were applied (resistance breakpoint 8 mg/L) due to the lack of CLSI recommendation. Serial dilutions were prepared ranging from 0.06 to 128 μ g/mL. MICs were read by naked eye, with the plates at the mirror, as the lowest concentration of an antibiotic that inhibited visible growth after 18 h at 37 °C. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as quality control strains. The isolates were classified as multidrug-resistant (MDR), extensively

drug-resistant (XDR), or pandrug-resistant (PDR), as described previously by Magiorakos et al. [10].

The double disk synergy test (DDST) [8] was carried out in the frames of routine laboratory analysis of the isolates in line with EUCAST guidelines. In brief, overnight culture of the test strain was adjusted to MacFarland 0.5 and inoculated on Mueller–Hinton (MH) agar (Biolife Italiana, Milan, Italy). A disk containing AMC (20/10 µg) was placed in the middle of the plate, and the surrounding ESC disks were placed 2 cm apart from the central disk. Distortion of the inhibition zone around the cephalosporin disk toward the central disk with clavulanic acid was considered suspicious of an ESBL. ESBL production was confirmed by CLSI combined disk test using disks with ESC alone or with the addition of clavulanic acid [9]. A difference of ≥ 5 mm between the zone diameters of either of the cephalosporin disks and their respective cephalosporin clavulanate disk is taken to be phenotypic confirmation of ESBL production. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as negative and positive control strains, respectively. AmpC β -lactamase overproduction or plasmid-mediated AmpC (p-AmpC) positivity was screened based on reduced susceptibility to FOX and confirmed by the combined disk test using ESC disks supplemented with cloxacillin [11]. A modified Hodge test (MHT) [12] and the carbapenem-inactivation method (CIM) were used to screen for the presence of carbapenemases [13]. MHT was carried out according to the previously published protocol. In brief, *E. coli* ATCC 25922, susceptible to carbapenems, was suspended in saline and inoculated on MacConkey agar (Biolife, Milan, Italy) to produce confluent growth. An MEM disk (10 µg) was placed in the center of the plate. The test isolates were streaked from the edge of the disk to the edge of the plate. Carbapenemase was suspected if the clover-leaf indentation of the indicator organism was observed toward the meropenem disc. Since MHT was found to have limited sensitivity, we applied an alternative method. CIM was performed according to the CLSI recommendations. In brief, a suspension of the test strain was adjusted to McFarland 0.5 (10^8 CFU/mL), and an MEM disk was placed in the suspension. The suspension was incubated for 2 h at 37 °C. *E. coli* ATCC 25922 was inoculated on MH agar, and the disk was placed in the middle of the plate. The plates were read after overnight incubation. The lack of an inhibition zone, decreased inhibition zone (<15 mm), or colonies within the inhibition zone indicated the presence of carbapenemase.

As a part of routine diagnostic procedures, the isolates with reduced susceptibility to carbapenems in the disk-diffusion test were subjected to the immunochromatographic OKNVI (OXA-48, KPC, NDM, VIM, IMP) (O.K.N.V.I. Resist-5, Coris Bioconcept, Gembloux, Belgium) [14] in the participating centers to determine the type of carbapenemase. Testing was performed as per the manufacturer's instructions. Briefly, one bacterial colony was taken from the 5% Sheep Blood agar (Biolife Italiana, Milan, Italy) and homogenized in the LY-A buffer. Three drops were then added to the sample well of each of the two cassettes (KPC/OXA-48 and NDM/VIM/IMP). Results were recorded after 15 min.

2.3. Conjugation

Conjugal transfer of ertapenem and cefotaxime resistance was attempted in mixed broth cultures at 35 °C. Donor and recipient strains were grown to the exponential stage, mixed in a ratio of 1:1, and incubated overnight at 37 °C. We used *E. coli* J65 resistant to sodium azide as the recipient [15]. The ESBL and carbapenemase-producing transconjugants were selected on MacConkey agar containing either ERT (0.5 mg/L) or CTX (2.0 mg/L) to inhibit the growth of the donors and sodium azide (100 mg/L) to suppress the recipient strain. Serial dilutions of donor and recipient strains and the mating mixture were prepared in order to determine the frequency of conjugation. It was determined relative to the number of donor cells.

2.4. Molecular Detection of Resistance Genes

The test was completed on all 30 isolates. An in-house DNA extraction was performed. A fresh bacterial colony was suspended in 100 µL of sterile, distilled water and boiled at 100 °C for 15 min. After centrifugation, the supernatant was used as a DNA template. Genotypic confirmation of resistance genes was completed by PCR targeting genes, conferring resistance to β-lactams, including broad and extended-spectrum β-lactamases (*bla*_{SHV}, *bla*_{TEM}, *bla*_{CTX-M}, and *bla*_{PER-1}) [16–19] and fluoroquinolone resistance genes (*qnrA*, *qnrB*, and *qnrS*) [20]. Genes encoding five phylogenetic clusters of CTX-M β-lactamases (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25) [21], plasmid-mediated AmpC β-lactamases [22], and carbapenemases belonging to the class A (*bla*_{KPC}), class B carbapenemases (*bla*_{VIM}, *bla*_{IMP}, and *bla*_{NDM}), and CHDL (*bla*_{OXA-48-like}) [23] were detected by multiplex PCR according to the previously published protocols. The primer sequences, annealing temperature, and amplicon sizes are provided in Table 1. The primers and the master mix were provided by Takara Bio Inc. San Jose, CA, USA. PCR products were separated by electrophoresis in a 1.5% agarose gel for 1 h at 100 V in 1× Tris-acetate-EDTA (TAE) buffer and stained with ethidium bromide. A 100 bp DNA ladder (Medical Intertrade, Zagreb, Croatia) was used as a molecular mass marker. DNA bands were visualized using a UV Transilluminator. Genetic context of *bla*_{CTX-M} genes was determined by PCR mapping with forward primer for *ISEcp1* and *IS26* combined with primer MA-3 (universal reverse primer for *bla*_{CTX-M} genes) [24]. Flanking regions of *bla*_{OXA-48} genes were analyzed by PCR using a forward primer for *IS1999* and a reverse primer for *bla*_{OXA-48} [25]. Amplicons were sequenced in both forward and reverse directions in Eurofins Genomic Service (<https://eurofinsgenomics.eu/>, accessed on 1 October 2025).

Table 1. Primers used in the study. Annealing temperature and the product length are provided.

Gene	Primer Sequence 5'-3'	Annealing Temperature	Product Length	Reference
<i>bla</i> _{TEM}	5'-ATG-AGT-ATT-CAA-CAT-TTC-CG-3' 5'-CCA-ATG-CTT-AAT-CAG-TGA-GG-3'	55	850	[16]
<i>bla</i> _{SHV}	5'-TTC-GCC-TGT-GTA-TTA-TCT-CCC-3' 5'-TTA-GCG-TTG-CCA-GTG-YTC-GAT-3'	58	1000	[17]
<i>bla</i> _{CTX-M}	5'-SCS-ATG-TGC-AGY-ACC-AGT-AA-3' 5'-CGC-CRA-TAT-GRT-TGG-TGG-TG-3'	55	550	[18]
<i>bla</i> _{CTX-M-1}	5'-AAA-AAT-CAC-TGC-GCC-AGT-TC-3' 5'-TTG-GTG-ACG-ATT-TTA-GCC-GC-3'	52	415	[21]
<i>bla</i> _{CTX-M-2}	5'-CGA-CGC-TAC-CCC-TGC-TAT-T-3' 5'-CCA-GCG-TCA-GAT-TTT-TCA-GG-3'	52	552	[21]
<i>bla</i> _{CTX-M-9}	5'-CAA-AGA-GAG-TGC-AAC-GGA-TG-3'3' 5'-ATT-GGA-AAG-CGT-TCA-TCA-CC-3'	52	205	[21]
<i>bla</i> _{CTX-M-8}	5'-TCG-CGT-TAA-GCG-GAT-GAT-GC-3' 5'-AAC-CCA-CGA-TGT-GGG-TAG-C-3'	52	666	[21]
<i>bla</i> _{CTX-M-25}	5'-GCA-CGA-TGA-CAT-TCG-GG-3' 5'-AAC-CCA-CGA-TGT-GGG-TAG-C-3'	52	327	[21]
<i>bla</i> _{MOX}	5'-GCT-GCT-CAA-GGA-GCA-CAG-GAT-3' 5'-CAC-ATT-GAC-ATA-GGT-GTG-GTG-C-3'	64	520	[22]
<i>bla</i> _{CMY}	5'-TGG-CCA-GAA-CTG-ACA-GGC-AAA-3' 5'-TTT-CTC-CTG-AAC-GTG-GCT-GGT-3'	64	462	[22]
<i>bla</i> _{DHA}	5'-AAC-TTT-CAC-AGG-TGT-GCT-GGG-T-3' 5'-CCG-TAC-GCA-TAC-TGG-CTT-TGC-3'	64	405	[22]

Table 1. Cont.

Gene	Primer Sequence 5'-3'	Annealing Temperature	Product Length	Reference
<i>bla_{ACC}</i>	5' AAC-AGC-CTC-AGC-AGC-CGG-TTA-3' TTC-GCC-GCA-ATC-ATC-CCT-AG-3'	64	346	[22]
<i>bla_{MIR}</i>	5' TCG-GTA-AAG-CCG-ATG-TTG-CGG 5' CTT-CCA-CTG-CGG-CTG-CCA-GTT-3'	64	302	[22]
<i>bla_{FOX}</i>	5' AAC-ATG-GGG-TAT-CAG-GGA-GAT-G-3' 5' CAA-AGC-GCG-TAA-CCG-GAT-TGG-3'	64	190	[22]
<i>bla_{IMP}</i>	5' GGAATAGAGTGGCTTAAYTCTC-3' GGTTTAAYAAAAACAACCACC-3'	52	232	[23]
<i>bla_{VIM}</i>	5'-GATGGTGTGTTGGTCGCATA-3' 5'-CGAATGCGCAGCACCAG-3'	52	390	[23]
<i>bla_{NDM}</i>	5'-GGTTTGGCGATCTGGTTTTTC-3' 5'-CGGAATGGCTCATCACGATC-3'	52	621	[23]
<i>bla_{KPC}</i>	5' CGTCTAGTTCTGCTGTCTTG-3' 5'-CTTGTCACTCCTTGTAGGCG-3'	52	798	[23]
<i>bla_{OXA-48}</i>	5'-GCGTGGTTAAGGATGAACAC-3' 5'-CATCAAGTTCAACCCAACCG-3'	52	438	[23]

2.5. Interarray Genotyping Kit CarbaResist

The genotyping of four randomly selected *P. stuartii* isolates was carried out with the microarray-based CarbaResist Genotyping Kit, in line with the manufacturer's instructions, version 1012012100004 (INTERARRAY, fzmB GmbH, Bad Langensalza, Germany). Genomic DNA was extracted from monoclonal overnight cultures with the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). The unfragmented DNA was then amplified by one primer for each target sequence (antisense) and was internally labeled with biotin dUTP. The obtained ssDNA products were then transferred into the ArrayWells to perform the hybridization. The wells contained 230 probes for the distinct genes encoding the most relevant carbapenemases, ESBL and AmpC, aminoglycoside (*aac*, *aad*, *aph*, *arm*, and *rmt*), sulfonamide (*sul*), trimethoprim (*dfr*), and fluoroquinolone resistance (*qnrA*, B, C, and S). The wells were washed to remove any unbound DNA, and horseradish peroxidase (HRP)-conjugated streptavidin was bound to all of the hybridized sections, resulting in dark spots on the chip due to an enzymatic reaction. The detection of these spots and data acquisition was carried out automatically by the INTER-VISION Reader.

2.6. Characterization of Plasmids

Plasmids were extracted with the Qiagen Plasmid Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. After staining with ethidium bromide, the DNA was visualized by ultraviolet light. PCR-based replicon typing (PBRT) [26] was applied to determine the plasmid content of the tested isolates. PBRT can be inefficient in identifying IncL/M plasmid types, and thus, we used an updated method designed to identify and distinguish between IncL and IncM plasmids [27]. Positive control strains for PBRT were kindly provided by Dr A. Carattoli (Istituto Superiore di Sanita, Rome, Italy).

2.7. Whole Genome Sequencing (WGS)

Nine representative isolates (six with OXA-48 and three with NDM) had their genomes sequenced as previously described [28]. Briefly, isolates were grown overnight on blood agar plates and had their DNA isolated using the DNeasy UltraClean Microbial Kit (Qiagen, Hilden, Germany). Sequencing libraries were prepared with the NEB Next® Ultra™ II FS

DNA Library Prep Kit for Illumina® (New England Biolabs, Frankfurt, Germany). The libraries were sequenced, 2 × 250 bp paired end, in an Illumina MiSeq (Illumina GmbH, Munich, Germany). The resulting Fasta files were assembled using the SKESA assembler (Version 2.4.0) as part of the Ridom SeqSphere+ software (Ridom GmbH, Münster, Germany, version 10.5.5) SKESA (version 2.4.0) and AMRFinderPlus (version 2024-12-18.1 (4.0)) are integrated into MBioSEQ Ridom version 10.5.5 (formerly known as SeqSphere) [29].

A core-genome MLST scheme (cgMLST) developed for *P. stuartii*, based on a core genome of 3079 target alleles (core) and 665 target alleles (accessory), was used to determine clonality. The 7-loci MLST sequence types were extracted from the assembled genome using PubMLST. Antimicrobial resistance genes were identified using NCBI AMRFinderPlus [30].

3. Results

3.1. Isolates and Patients

In total, 30 non-duplicate (one per patient) isolates were collected: 13 from ASPH (11 from Sv. Ivan Zelina Hospital and 2 from outpatients) and CHCR, respectively, and 2 from UHS and UHCSM, respectively. There were 22 *P. stuartii* and 8 *P. rettgeri* isolates. The patient's age ranged from 32 to 95 (mean value 74.8), and the study included 21 females and 9 males. Most of the isolates originated from the urinary tract (14 from urinary catheter and 10 from midstream urine). All patients with urinary tract infection (UTI) had $>10^5$ CFU/mL of *Providencia* spp. in a pure culture and pyuria (>5 white blood cells/high power field) or a positive test for leukocyte esterase. There was one isolate from blood culture and wound swab, respectively, and four from surveillance cultures (rectum swab and endotracheal aspirate). Twenty-six patients had an infection (urinary tract, wound, and bloodstream infection), whereas four were only colonized. The clinical and epidemiological data are summarized in Table 2.

3.2. Antimicrobial Susceptibility Testing and Phenotypic Tests for Detection of ESBLs, AmpC β -Lactamases, and Carbapenemases

The isolates were fully resistant to CXM, CAZ, CRO, CTX, FEP, TZP, IMI, MEM, ERT, SMX, and CHL, in addition to the antibiotics to which they are intrinsically resistant (AMX, TET, and GM), with MICs of most cephalosporins exceeding 128 mg/L, as shown in Table 2. Moderate resistance rates were observed for AMI (40%) (Table 2). One isolate tested resistant to FOX. There was a marked difference in the AMI-resistant rates between OXA-48 and NDM-producing organisms (73% vs. 0%). Interestingly, 47% of the isolates were susceptible to GM in spite of the intrinsic resistance of *Providencia* spp. According to initial DDT, all isolates were resistant to AMX, AMC, TZP, CN, CXM, CAZ, CTX, FEP, IMI, MEM, ERT, and CIP. The rates of AMI, CZA, and FDC resistance were 40% (12/30), 50% (15/30), and 37% (11/30), respectively. The immunochromatographic test demonstrated OXA-48 and NDM carbapenemases in 15 isolates each. KPC carbapenemases were not detected. Phenotypic tests for ESBLs were positive in all OXA-48 and one NDM-positive organism (16 isolates). Screening for p-AmpC was positive in one isolate, which was confirmed as p-AmpC positive by an inhibitor-based test with cloxacillin. The isolates were categorized as XDR. OXA-48-producing isolates were susceptible only to CZA, whereas NDM producers tested susceptible to FDC and the majority to AMI (93%) $n = 14$ as well. MHT successfully identified both OXA-48 and NDM carbapenemase. In contrast, the CIM test demonstrated high sensitivity only for OXA-48 (93%, $n = 14$) but very poor performance with NDM producers (20%, $n = 3$).

Table 2. Clinical data, antibiotic resistance profile, and β -lactamase content of *Providencia* spp. isolates.

	PROTOCOL NUMBER AND SPECIES	AGE AND GENDER	SPECIMEN	CLINICAL DIAGNOSIS	OUTCOME	CENTER AND HOSPITAL WARD	TZP	CAZ	CTX	CRO	FEP	IMI	MEM	AMI	CIP	β -LACTAMASE CONTENT
1	45767/2-24 PS	93 F	Urine- catheter	UTI	D	OUTPATIENT	≥ 128 (R)	≥ 128 (R)	≥ 128 (R)	32 (R)	32 (R)	16 (R)	16 (R)	16 (S)	32 (R)	NDM, CTX-M-15
2	39520/2-24 PS	80 M	Urine- catheter	UTI	D	OUTPATIENT	128 (R)	≥ 128 (R)	≥ 128 (R)	64 (R)	≥ 128 (R)	8 (R)	8 (R)	16 (S)	64 (R)	NDM
3	48059/2-24 PS	86 F	Urine- catheter	Dementia UTI	NA	SV. IVAN GAERIATRICS	128 (R)	16 (R)	≥ 128 (R)	64 (R)	≥ 128 (R)	8 (R)	16 (R)	≥ 128 (R)	128 (R)	OXA-48, CTX-M-15 TEM
4	49097/2-24 PS	81 F	Urine- catheter	Dementia UTI	NA	SV. IVAN GAERIATRICS	128 (R)	4 (R)	≥ 128 (R)	≥ 128 (R)	32 (R)	16 (R)	32 (R)	≥ 128 (R)	≥ 128 (R)	OXA-48, CTX-M-15 TEM
5	46920/2-24 PS	68 F	Urine- catheter	Dementia UTI	NA	SV. IVAN GAERIATRICS	128 (R)	4 (R)	≥ 128 (R)	8 (R)	64 (R)	8 (R)	32 (R)	≥ 128 (R)	≥ 128 (R)	OXA-48, CTX-M-15 TEM
6	30022/2-24 PS	75 F	Urine- catheter	Dementia UTI	NA	SV. IVAN GAERIATRICS	≥ 128 (R)	4 (R)	≥ 128 (R)	32 (R)	128 (R)	16 (R)	64 (R)	≥ 128 (R)	≥ 128 (R)	OXA-48, CTX-M-15 TEM
7	19729/2-24 PS	82 F	Urine- catheter	Dementia UTI	NA	SV. IVAN GAERIATRICS	≥ 128 (R)	4 (R)	≥ 128 (R)	≥ 128 (R)	32 (R)	32 (R)	32 (R)	≥ 128 (R)	≥ 128 (R)	OXA-48, CTX-M-15
8	47950/2-24 PS	87 F	Urine- catheter	Dementia UTI	NA	SV. IVAN GAERIATRICS	≥ 128 (R)	8 (R)	≥ 128 (R)	≥ 128 (R)	64 (R)	16 (R)	32 (R)	≥ 128 (R)	64 (R)	OXA-48, CTX-M-15 TEM
9	28170/2-24 PS	83 F	Urine- catheter	Dementia UTI	NA	SV. IVAN GAERIATRICS	≥ 128 (R)	4 (R)	≥ 128 (R)	≥ 128 (R)	16 (R)	16 (R)	32 (R)	≥ 128 (R)	64 (R)	OXA-48, CTX-M-15 TEM
10	50091/2-24 PS	89 F	Urine- catheter	Dementia UTI	NA	SV. IVAN GAERIATRICS	≥ 128 (R)	8 (R)	≥ 128 (R)	32 (R)	32 (R)	32 (R)	32 (R)	≥ 128 (R)	64 (R)	OXA-48, CTX-M-15 TEM
11	50257/2-24 PS	95 F	Urine- catheter	Dementia UTI	NA	SV. IVAN GAERIATRICS	≥ 128 (R)	8 (R)	≥ 128 (R)	64 (R)	128 (R)	64 (R)	128 (R)	≥ 128 (R)	≥ 128 (R)	OXA-48, CTX-M-15 TEM
12	48272/-24 PS	95 F	Urine- catheter	Dementia UTI	NA	SV. IVAN GAERIATRICS	≥ 128 (R)	128 (R)	≥ 128 (R)	16 (R)	16 (R)	16 (R)	32 (R)	≥ 128 (R)	128 (R)	OXA-48, CTX-M-15
13	30472/2-24 PS	95 F	Urine- catheter	Dementia UTI	NA	SV. IVAN GAERIATRICS	≥ 128 (R)	4 (R)	≥ 128 (R)	32 (R)	64 (R)	16 (R)	32 (R)	≥ 128 (R)	≥ 128 (R)	OXA-48, CTX-M-15 TEM
14	UG3084 PS	55 M	Urine- catheter	Chronic myeloid leukemia	S	UHS hematology	≥ 128 (R)	4 (R)	≥ 128 (R)	64 (R)	64 (R)	16 (R)	32 (R)	16 (S)	128 (R)	OXA-48, CTX-M-15
15	UG48005 PS	53 M	Urine	Non-Hodkin lymphoma	S	UHS hematology	≥ 128 (R)	32 (R)	≥ 128 (R)	≥ 128 (R)	64 (R)	8 (R)	32 (R)	8 (S)	64 (R)	OXA-48, CTX-M-15

Table 2. Cont.

	PROTOCOL NUMBER AND SPECIES	AGE AND GENDER	SPECIMEN	CLINICAL DIAGNOSIS	OUTCOME	CENTER AND HOSPITAL WARD	TZP	CAZ	CTX	CRO	FEP	IMI	MEM	AMI	CIP	β-LACTAMASE CONTENT
16	68511 PS	85 F	Urine	UTI	D	CHCR	≥128 (R)	≥128 (R)	≥128 (R)	≥128 (R)	32 (R)	32 (R)	32 (R)	16 (S)	4 (R)	NDM
17	69188 PS	70 M	Urine	Bowel cancer, UTI	S	CHCR	≥128 (R)	≥128 (R)	≥128 (R)	≥128 (R)	128 (R)	32 (R)	32 (R)	32 (R)	4 (R)	NDM
18	60953 PS	83 F	Urine	Cerebral stroke, UTI	D	CHCR	≥128 (R)	≥128 (R)	≥128 (R)	≥128 (R)	128 (R)	32 (R)	32 (R)	16 (S)	4 (R)	NDM
19	62849PR	74 F	Urine	Diabetes mellitus, UTI	D	CHCR ICU	≥128 (R)	≥128 (R)	≥128 (R)	≥128 (R)	128 (R)	32 (R)	32 (R)	16 (S)	8 (R)	NDM
20	63703 PR	60 M	Urine	Convulsions UTI	S	CHCR neurology	128 (R)	≥128 (R)	≥128 (R)	≥128 (R)	128 (R)	32 (R)	32 (R)	16 (S)	4 (R)	NDM
21	61738 PR	77 F	BC	UTI BSI	S	CHCR surgery	128 (R)	≥128 (R)	≥128 (R)	≥128 (R)	128 (R)	32 (R)	32 (R)	16 (S)	4 (R)	NDM
22	54062 PR	78 F	Rectum swab	Cranial trauma	S	CHCR surgery	≥128 (R)	≥128 (R)	≥128 (R)	≥128 (R)	64 (R)	32 (R)	32 (R)	16 (S)	4 (R)	NDM
23	35649 PR	68 F	Urine	Acute respiratory failure	S	CHCR ICU	≥128 (R)	≥128 (R)	≥128 (R)	≥128 (R)	128 (R)	16 (R)	32 (R)	16 (S)	128 (R)	NDM
24	42237 PR	77 F	Urine	Cerebral stroke, UTI	D	CHCR emergency	64 (R)	≥128 (R)	≥128 (R)	64 (R)	128 (R)	32 (R)	128 (R)	16 (S)	4 (R)	NDM
25	32488 PS	52 M	Rectum swab	UTI BSI	S	CHCR ICU	≥128 (R)	≥128 (R)	≥128 (R)	≥128 (R)	≥128 (R)	32 (R)	32 (R)	16 (S)	4 (R)	NDM
26	29664 PS	88 M	Urine	Acute respiratory failure, UTI	S	CHCR emergency	≥128 (R)	64 (R)	≥128 (R)	≥128 (R)	≥128 (R)	16 (R)	32 (R)	16 (S)	64 (R)	NDM
27	22994 PR	81 F	Urine	UTI, BSI	D	CHCR emergency	≥128 (R)	128 (R)	≥128 (R)	≥128 (R)	≥128 (R)	16 (R)	32 (R)	16 (S)	64 (R)	NDM
28	53151 PR	52 F	Tracheal aspirate	Brain damage, pleural effusion	H	CHCR ICU	64 (R)	128 (R)	≥128 (R)	64 (R)	32 (R)	16 (R)	32 (R)	8 (S)	4 (R)	NDM
29	14811 PS	44 M	Wound swab	Wound infection	S	UHSM Surgical ICU	≥128 (R)	≥128 (R)	32 (R)	16 (R)	128 (R)	32 (R)	32 (R)	≥128 (R)	≥128 (R)	OXA-48, CTX-M-15 TEM
30	20585 PS	32 M	Tracheal aspirate		S	UHSM Surgical ICU	≥128 (R)	≥128 (R)	≥128 (R)	≥128 (R)	64 (R)	8 (R)	64 (R)	4 (S)	32 (R)	OXA-48, CTX-M-15

Abbreviations: TZP—piperacillin–tazobactam; CAZ—ceftazidime; CTX—cefotaxime; CRO—ceftriaxone; FEP—cefepime; IMI—imipenem; MEM—meropenem; AMI—amikacin; CIP—ciprofloxacin; D—death; S—survival; NA—not applicable; H—still in the hospital; M—male; F—female; PS—*P. stuartii*; PR—*P. rettgeri*; UTI—urinary tract infection; BSI—bloodstream infection; S—susceptible; R—resistant.

3.3. Conjugation

The isolates failed to transfer either imipenem or cefotaxime resistance to the *E. coli* recipient strain.

3.4. Molecular Detection of Resistance Genes

PCR confirmed the results of the preliminary immunochromatographic testing. *bla*_{OXA-48-like} and *bla*_{NDM} genes were found in 15 isolates, respectively. *bla*_{KPC} genes were not detected. All 16 isolates phenotypically positive for an ESBL yielded a PCR product with primers specific for the *bla*_{CTX-M} gene, belonging to the phylogenetic cluster 1. *bla*_{TEM} genes were identified in 11 isolates harboring OXA-48, all from Zagreb (Table 2). The cefoxitin-resistant strain turned out to be positive for the *bla*_{CMY} gene. Sequencing of *bla*_{CTX-M} genes revealed CTX-M-15 allelic variant. The ISEcp insertion element was associated with *bla*_{CTX-M} genes. The IS1999 was not found.

3.5. Interarray CarbaResist Kit

An Interarray CarbaResist Kit identified identical resistance gene content in four selected OXA-48-producing organisms: *bla*_{CTX-M-15}, *bla*_{TEM}, and *bla*_{OXA-10} for β -lactam resistance (Table 3). There was a plethora of aminoglycoside resistance genes: *aadA1* and *aadA2* encoding adenylyltransferases and *arm* gene for 16S rRNA methylase present in all isolates, with one isolate harboring *aac*(6'')Ib for acetyltransferase as well. Sulfonamide resistance was mediated by *sul1* and *sul2* genes, generating dihydropteroate synthetase, whereas *dfrA12* and *dfrA14* were linked to dihydrofolate reductase, an enzyme responsible for trimethoprim resistance (Table 3). All four isolates were positive for class 1 integron.

Table 3. Interarray CarbaResist Kit analysis of selected isolates. PS: *P. stuartii*.

Isolate No	Res. Phenotype	β -Lactam	Aminoglycoside	Sulfonamide	Trimethoprim	Integrase Gene
PS 9	TZP, CAZ, CTX, CRO, FEP, GM, AMI, CIP	ISEcp- <i>bla</i> _{CTX-M-15} <i>bla</i> _{TEM} <i>bla</i> _{OXA-10} <i>bla</i> _{OXA-48}	<i>aadA1</i> <i>aadA2</i> <i>armA</i>	<i>sul1</i> <i>sul2</i>	<i>dfrA12</i> <i>dfrA14</i>	<i>intl1</i>
PS 10	TZP, CAZ, CTX, CRO, FEP, GM, AMI, CIP	ISEcp- <i>bla</i> _{CTX-M-15} <i>bla</i> _{TEM} <i>bla</i> _{OXA-1} <i>bla</i> _{OXA-10} <i>bla</i> _{OXA-48}	<i>aac</i> (6'') <i>aac</i> (6'')Ib <i>aadA1</i> <i>aadA2</i> <i>armA</i>	<i>sul1</i> <i>sul2</i>	<i>dfrA12</i> <i>dfrA14</i>	<i>intl1</i>
PS 11	TZP, CAZ, CTX, CRO, FEP, GM, AMI CIP	ISEcp- <i>bla</i> _{CTX-M-15} <i>bla</i> _{TEM} <i>bla</i> _{OXA-10} <i>bla</i> _{OXA-48}	<i>aadA1</i> <i>aadA2</i> <i>armA</i>	<i>sul1</i> <i>sul2</i>	<i>dfrA12</i> <i>dfrA14</i>	<i>intl1</i>
PS 13	TZP, CAZ, CTX, CRO, FEP, GM, AMI CIP	ISEcp- <i>bla</i> _{CTX-M-15} <i>bla</i> _{TEM} <i>bla</i> _{OXA-10} <i>bla</i> _{OXA-48}	<i>aadA1</i> <i>aadA2</i> <i>armA</i>	<i>sul1</i> <i>sul2</i>	<i>dfrA12</i> <i>dfrA14</i>	<i>intl1</i>

3.6. WGS

There were two allelic variants of *bla*_{NDM} genes: *bla*_{NDM-1} and *bla*_{NDM-5} and only one *bla*_{OXA-48} variant. One NDM-positive organism harbored the plasmid-mediated AmpC, β -lactamase *bla*_{CMY-16}, in addition to a carbapenemase. WGS identified additional phosphorylases (*aph*(3'')-Ib and *aph*(6)-Id) in six isolates, not detected with the Interarray CarbaResist Kit, which are in line with elevated MICs of AMI (Table 4). Genes for *aadA36* adenylylase were present in all isolates, while *aadA1* and *aadA2* were found only in five OXA-48-producing organisms (Table 4). The *sul1* gene was present in all but one isolate, while *sul2* was carried by six isolates. Isolates harbored four allelic variants of *dfr* genes: *dfrA1*,

dfrA10, and *dfrA14*. *dfrA1* and *dfrA10* were found only in NDM-positive organisms, while *dfrA12* was identified in five OXA-48 producers. *Tet(A)* and *tet(B)* were responsible for TET resistance, with *tet(B)* being present in all tested isolates, while *catA3* and *catA5* encoded chloramphenicol acetyltransferase, causing CHL resistance (Table 4). One OXA-48 positive isolate tested positive for *aac(6')-Ib-cr5*, which is associated with combined aminoglycoside and fluoroquinolone resistance (Table 4).

Table 4. WGS of selected isolates. PS: *P. stuartii*.

Isolate Number	β -Lactam	Aminoglycoside	Sulfonamide	Trimethoprim	Tetracycline	Chloramphenicol	ST	Cluster	Plasmid Replicon
PS 2	<i>bla</i> _{CMY-16} <i>bla</i> _{NDM-1}	<i>aac(2')-Ia</i> <i>aadA36</i> <i>aph(3'')-Ib</i> <i>aph(6)-Id</i>	<i>sul1</i> <i>sul2</i>	<i>dfrA1</i>	<i>tet(A)</i> <i>tet(B)</i>	<i>catA3</i>	ST5	singleton	IncC
PS 4	<i>bla</i> _{TEM-1} <i>bla</i> _{OXA-10} <i>bla</i> _{CTX-M-15} <i>bla</i> _{OXA-48}	<i>aac(2')Ia</i> <i>aadA1</i> <i>aadA2</i> <i>aadA36</i> <i>aph(3'')-Ib</i> <i>aph(6)-Id</i>	<i>sul1</i> <i>sul2</i>	<i>dfrA12</i> <i>dfrA14</i>	<i>tet(B)</i>	<i>catA3</i> <i>cmlA5</i>	ST22	1	IncC
PS 7	<i>bla</i> _{TEM-1} <i>bla</i> _{OXA-10} <i>bla</i> _{CTX-M-15} <i>bla</i> _{OXA-48}	<i>aac(2')Ia</i> <i>aadA1</i> <i>aadA2</i> <i>aadA36</i> <i>aph(3'')-Ib</i> <i>aph(6)-Id</i>	<i>sul1</i> <i>sul2</i>	<i>dfrA12</i> <i>dfrA1</i>	<i>tet(B)</i>	<i>catA3</i> <i>cmlA5</i>	ST22	1	IncC
PS 8	<i>bla</i> _{TEM-1} <i>bla</i> _{OXA-10} <i>bla</i> _{CTX-M-15} <i>bla</i> _{OXA-48}	<i>aac(2')Ia</i> <i>aadA1</i> <i>aadA2</i> <i>aadA36</i> <i>aph(3'')-Ib</i> <i>aph(6)-Id</i>	<i>sul1</i> <i>sul2</i>	<i>dfrA12</i> <i>dfrA14</i>	<i>tet(B)</i>	<i>catA3</i> <i>cmlA5</i>	ST22	1	IncC
PS 9	<i>bla</i> _{TEM-1} <i>bla</i> _{OXA-10} <i>bla</i> _{CTX-M-15} <i>bla</i> _{OXA-48}	<i>aac(2')Ia</i> <i>aadA1</i> <i>aadA2</i> <i>aadA36</i> <i>aph(3'')-Ib</i> <i>aph(6)-Id</i>	<i>sul1</i> <i>sul2</i>	<i>dfrA12</i> <i>dfrA14</i>	<i>tet(B)</i>	<i>catA3</i> <i>cmlA5</i>	ST22	1	IncC
PS 10	<i>bla</i> _{TEM-1} <i>bla</i> _{OXA-10} <i>bla</i> _{CTX-M-15} <i>bla</i> _{OXA-48}	<i>aac(2')Ia</i> <i>aac(6')-Ib-cr56</i> <i>aadA1</i> <i>aadA2</i> <i>aadA36</i> <i>aph(3'')-Ib</i> <i>aph(6)-Id</i>	<i>sul1</i> <i>sul2</i>	<i>dfrA12</i> <i>dfrA14</i>	<i>tet(B)</i>	<i>catA3</i> <i>cmlA5</i>	ST22	singleton	IncC
PS 14	<i>bla</i> _{OXA-48}	<i>aac(2')Ia</i> <i>aadA36</i>			<i>tet(B)</i>	<i>catA3</i>	ST22	singleton	IncC
PS 17	<i>bla</i> _{NDM-5}	<i>aac(2')Ia</i> <i>aadA36</i>	<i>sul1</i>	<i>dfrA10</i>	<i>tet(B)</i>	<i>catA3</i>	ST3	2	IncC
PS18	<i>bla</i> _{NDM-5}	<i>aac(2')Ia</i> <i>aadA36</i>	<i>sul1</i>	<i>dfrA10</i>	<i>tet(B)</i>	<i>catA3</i>	ST3	2	IncC

Molecular typing reveals that there were six ST22 isolates, two ST3 isolates, and one ST5 isolate (Table 4). cgMLST analysis reveals that there are two transmission clusters and three singletons (Figure 1). One cluster of four isolates was ST22, all from Sv. Ivan Zelina hospital in Zagreb, also closely related to the other two ST22 isolates, but with 17–29 alleles outside the threshold of 15 alleles. The two ST3 isolates differed in three alleles.

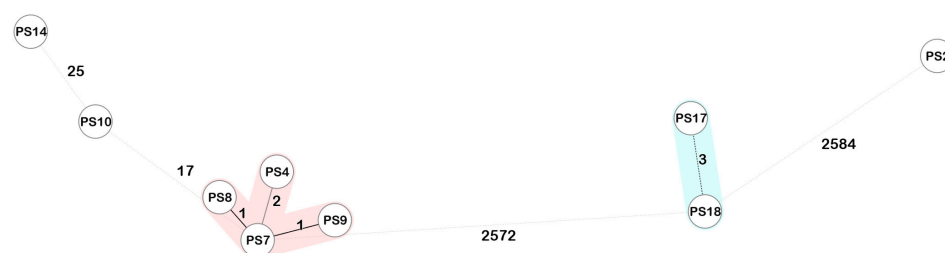


Figure 1. cgMLST minimum spanning tree showing genetic relatedness between the isolates based on 3744 gene targets (3079 core and 665 accessory). Numbers in the nodes are the isolate numbers, and the numbers between the nodes are the allele differences. Transmission threshold is 15 alleles.

3.7. Plasmid Analysis

PBRT tested negative for plasmid replicons, but WGS identified IncC plasmid replicon in five of the sequenced isolates: the OXA-48-positive isolates PS7, PS9, PS8, PS4, and PS10 and the NDM-1-positive isolate PS2. The two NDM-5 isolates have a Col3M plasmid. Short-read sequencing data are unreliable for plasmid analysis; however, MOB-suite predicts that only the isolate PS14 has a carbapenemase (OXA-48) encoded on a plasmid.

4. Discussion

The overuse of carbapenems has driven the rise of CRPS in Europe, including Croatia. The rise of NDM-producing CRPS in Europe was noticed following the onset of war in Ukraine and the subsequent arrival of refugees in the European Union [1]. NDM was previously found among CRPS in Romania [31,32], Bulgaria [33], and Portugal [34], with some studies pre-dating the current conflict. The isolates from Bulgaria harbored additional CMY-4 p-AmpC. In our study, a CMY-16 allelic variant was found as an additional β -lactamase in one NDM-1-producing organism. It seems that the spread of CRPS in Europe occurred first in the Iberian Peninsula and later in Eastern Europe. Outside Europe, NDM-positive CRPS were described in Afghanistan much earlier, back in 2012 [35], and later in Israel [36]. In Greece, carbapenem resistance was due to another family of MBLs—VIM [37]. In contrast, in South America, KPC-2 was found responsible for carbapenem resistance in CRPS [38], with additional CTX-M-2 and CTX-M-9 contributing to ESC resistance. IMP variants are the rarest among CRPS and are reported only in Mexico, alongside OXA-24 and OXA-58, CHDLs, more associated with *Acinetobacter baumannii* [39], demonstrating the ability of *P. stuartii* to acquire resistance determinants from other Gram-negative bacteria. Coproduction of OXA-48 and NDM-1 was described recently in PDR *P. rettgeri* isolated in Turkey [40], showing the amazing capacity of the species to accumulate resistance traits. Thus, resistance determinants conferring carbapenem resistance depend on the local epidemiology. It seems that the spread of CRPS occurred in countries with a high rate of carbapenemase-producing *Enterobacteriales* (CPE), indicating the probable transfer of *bla*_{CARB} genes from *K. pneumoniae* and *E. coli*. Croatia has a high rate of CPE of approximately 6% [41], and excessive colistin usage in the hospitals provides a survival advantage to intrinsically colistin-resistant organisms like *Proteus* spp. and *Providencia* spp. [42]. With the growing burden of CPE and carbapenem-resistant *A. baumannii* (CRAB), colistin therapy as the salvage treatment is on the rise. The urinary tract, particularly urinary catheters, was shown to be the dominant source of CRPS. This is in line with the capacity of this genus to produce biofilm [1]. Interestingly, *P. rettgeri* was identified only in CHCR, whereas in the other three centers, only *P. stuartii* was found to harbor carbapenemase-encoding genes.

The main finding presented in this study is the equal presence of NDM and OXA-48 carbapenemases among CRPS in Croatia. All our isolates were shown to be XDR. OXA-48-positive isolates were susceptible to CZA, whereas NDM producers demonstrated sensitivity to FDC and the majority to AMI. Interestingly, FDC resistance was noted only

among OXA-48-producing organisms from Zagreb. There was no FDC resistance among MBL-producing organisms from Rijeka. Thus, FDC could be considered a good therapeutic option for infections with NDM producers. However, there is also an issue with the standard used. According to EUCAST, which is used in Europe, the majority of OXA-48-positive isolates were resistant, but if CLSI criteria were applied with a lower breakpoint, all isolates would be assessed as susceptible. The isolates exhibited very variable MICs of carbapenems and ESC, but within the resistance range. This variability may be due to differential expression of ESBL and carbapenemase-encoding genes, probably due to mutations in the promotor region of the genes or variable gene copy numbers. In general, the level of ESC resistance was much higher among OXA-48 producers, likely due to the co-production of an additional ESBL. Among NDM-positive organisms, there was discordance between high MICs of MEM, IMI, and FEP—indicating resistance—and large inhibition zones, falling within the susceptible or intermediate susceptible range.

MHT proved to be a reliable phenotypic testing option for both OXA-48 and NDM, in contrast to CIM, which yielded a lot of false negative results. NDM is a membrane-bound lipoprotein connected to the outer membrane. Therefore, it inhibits the release of NDM carbapenemase into the extracellular medium, unlike soluble MBLs [43].

Phenotypic tests proved the production of an ESBL in all OXA-48- and one NDM-positive isolate. PCR identified the gene encoding CTX-M belonging to clonal lineage 1 with CTX-M-15 as the only allelic variant. A small outbreak of urinary tract infections with CTX-M-15-producing *P. stuartii* was reported in the University Hospital Centre Split in 2012 [44]. In the present study, *bla*_{CTX-M} genes were preceded by the *ISEcp* insertion element, important for the mobilization of the ESBL coding gene. One isolate positive for NDM coharbored CMY-16. This type of p-AmpC was previously identified among *Proteus mirabilis* isolates from University Hospital Split [45]. Additional ESBLs and p-AmpC are often reported in CRPS [46], contributing to ESC resistance. Since OXA-48 does not hydrolyze cephalosporins, ESC resistance was attributed to CTX-M-15, the variant that efficiently hydrolyzes all cephalosporins. The fact that IS1999 was not identified by PCR mapping indicates that *bla*_{OXA-48} genes in CRPS were located in a different genetic environment compared to *K. pneumoniae* and *E. coli* [25].

The majority of isolates originated from within the hospital setting, but two NDM-1 isolates were isolated from outpatients, indicating the spread of the CRPS to the community. Community-acquired NDM-positive isolates harbored additional β -lactam resistance determinants such as ESBL or p-AmpC, while MBL genes were the sole β -lactam resistance genes among hospital isolates. Despite the presence of aminoglycoside resistance genes, all NDM-producing organisms were susceptible to amikacin, in contrast to OXA-48 producers, which, in the majority of cases, displayed high-level resistance with MICs above 128 mg/L. The phenomenon could be linked to the lack of *aph* genes among the majority of NDM-positive isolates. Even the MICs of gentamicin, which is intrinsically ineffective against *Providenciae*, were within the susceptible range among NDM-positive organisms. The majority of isolates did not carry acquired fluoroquinolone resistance genes, suggesting that chromosomal mutations of *gyrA* and *parC* genes may explain elevated MICs of ciprofloxacin and lack of inhibition zones around norfloxacin and levofloxacin disks. Only one strain tested positive for the acquired aminoglycoside and fluoroquinolone resistance determinant *aac(6')-Ib-cr5*. High level of ciprofloxacin resistance with MIC values exceeding 128 mg/L was linked to OXA-48 carbapenemase producers, whereas the majority of NDM producers exhibited low-level resistance with MIC values around 4 mg/L. The presence of *sul* and *dfr* genes was in line with phenotypic resistance to SMX. Discrepancies between Interarray CarbaResist Kit and WGS were observed for isolate 9, which lacked the *armA* gene in WGS, and isolate 10, for which *bla*_{OXA-1}, *aac(6'')*, and *armA* genes were found by

the Interarray CarbaResist Kit, but not by WGS. This could be attributable to the loss of resistance genes when the isolates were subcultured without selection pressure.

The genetic relatedness was evaluated by WGS, and two transmission clusters were found. This suggests clonal cross-transmission of isolates and the possibility of transmission between patients and medical staff by hand colonization and by environmental contamination. It seems that the isolates belonging to ST3 and ST5 carried NDM coding genes, while *bla*_{OXA-48} genes were carried by ST22 isolates. In Romania, NDM-positive organisms belonged to ST28, ST46, and ST233 [31].

Although the IncC plasmid replicon was found in five OXA-48-positive isolates subjected to WGS, there is no proof that it harbors carbapenemase-encoding genes for several reasons: the failure to transfer resistance via conjugation experiments and the MOB-suite prediction. However, these data were generated by short-read sequencing, which is not always a reliable predictor of plasmid-located resistance genes. The association of IncC plasmid and *bla*_{NDM} genes was described in Romanian and Italian isolates [31,47]. Due to the problems with resistance transfer, we could not determine if the spread of carbapenemase-producing CRPS was due to vertical expansion of related isolates or horizontal transmission of IncC plasmids harboring *bla*_{CARB} genes. NDM carbapenemase-producing isolates are often associated with hospital outbreaks, which are difficult to control. However, in our study, the endemic presence of CRPS in the participating centers, over a prolonged time, was noticed. Regarding the therapeutic options, CZA appears effective against OXA-48-positive isolates, whereas FDC and AMI demonstrated activity against NDM-producing isolates.

The strength of the present study is the large-scale susceptibility data obtained according to the standardized protocols. In addition, we tested antimicrobial susceptibility to many last resort antibiotics. However, this study has several limitations. The participating centers do not cover all regions in Croatia, for example, Slavonia. Isolates were collected retrospectively. Systematic screening for carriage of CRPS was not performed. Moreover, the study used a relatively small number of isolates, and the genetic comparison between isolates was not carried out for all isolates. Furthermore, aminoglycoside, sulfonamide, trimethoprim, tetracycline, and chloramphenicol resistance genes were not determined for the whole collection of isolates.

5. Conclusions

This study found equal presence of OXA-48 and NDM carbapenemases in *Providencia* spp. In other *Enterobacterales* in Croatia, OXA-48 is the dominant carbapenemase, with only sporadic occurrence of NDM. This study revealed regional differences in the prevalence of carbapenemase types: OXA-48 was dominant in Zagreb and Split, while NDM prevailed in Rijeka. Identification of NDM-1 in the outpatients' samples indicates diffusion of this resistance determinant to the community. Resistance phenotypes did not differ between the two outpatients and the hospital isolates, indicating diffusion of the nosocomial isolates into the community. Spread of CRPS poses a serious therapeutic challenge for clinicians due to the limited therapeutic options and lack of new antibiotics. Moreover, identification of carbapenemases in CRPS may be a challenge because of the poor performance of phenotypic tests. Future studies on a larger collection of isolates should elucidate the modes and routes of spread of these increasingly resistant pathogens.

The effective use of molecular epidemiology methods such as WGS provides useful data for elucidating the transmission pathways of this rare, but increasingly important hospital pathogen. *Providencia* spp., in spite of being a rare pathogen, should be included in the surveillance studies across the medical centers in Croatia. Since there was a clonal expansion detected in Sv. Ivan Zelina hospital in Zagreb, more attention should be paid to hospital hygiene measures to avoid cross-infections.

Author Contributions: Conceptualization: J.V. and B.B.; methodology: B.B., J.L., G.Z. and H.C.; validation: B.B. and J.V. formal analysis: B.B., J.V. and P.G.H.; investigation: B.B., P.G.H., H.C., G.Z., J.L., T.B. and R.A.d.P.; resources: B.B., A.G. and J.V.; data curation: S.S.; M.B.-Š., M.A., J.L., V.T. and T.B.; writing—original draft: B.B.; writing—review and editing: J.V., A.G., P.G.H., G.Z. and J.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by “Extended-spectrum β -lactamases, plasmid-mediated β -lactamases, and carbapenemases in *Proteus mirabilis*” grant, number 10106-24-1294, by the University of Zagreb and by the “Molecular epidemiology of multidrug-resistant uropathogens and their dissemination potential in the community” grant, number IP-2025-6757, by the Croatian Science Foundation.

Institutional Review Board Statement: Permission for this study was obtained from the Ethical Commission of the Dr Andrija Štampar Teaching Institute of Public Health, Zagreb, Croatia (Permission No. 641-01/25-01/1, dated 20 February 2025).

Informed Consent Statement: Patient consent was waived. It was not necessary because the study was not conducted on human subjects; only bacterial isolates were analyzed. This is an in vitro study.

Data Availability Statement: All raw sequencing reads were submitted to the European Nucleotide Archive (<https://www.ebi.ac.uk/ena/>, accessed on 14 October 2025) with the Project Number PRJEB90535.

Acknowledgments: We thank Marina Nađ and Mislav Kasalo for technical assistance.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript: AMC—amoxycillin/clavulanic acid; TZP—piperacillin-tazobactam; CXM—cefuroxime; CAZ—ceftazidime; CTX—cefotaxime; CRO—ceftriaxone; FEP—cefepime; IMI—imipenem; MEM—meropenem; GM—gentamicin; AMI—amikacin; CIP—ciprofloxacin; COL—colistin; CZA—ceftazidime-avibactam; FDC—cefiderocol; MHT—modified Hodge test; CIM—carbapenem inactivation method; ESC—expanded-spectrum cephalosporins; MIC—minimum inhibitory concentration; DDST—double disk synergy test; MBL—metallo- β -lactamase; CHDL—carbapenem-hydrolyzing oxacillinase; UHS—University Hospital Split; UHCSM—University Hospital Center Sestre Milosrdnice; ASPH—Dr Andrija Štampar Public Health Institute; MHA—Mueller-Hinton agar; ESBL—extended-spectrum β -lactamase; p-AmpC—plasmid-mediated AmpC β -lactamase; ST—sequence type; ETA—endotracheal aspirate; PCR—polymerase chain reaction; WGS—whole genome sequencing; MHB—Mueller-Hinton broth. CHL—chloramphenicol.

References

1. Witteven, S.; Hendrickx, A.P.A. *Providencia stuartii*. *Trends. Microbiol.* **2025**, *33*, 810–811. [CrossRef]
2. Bradford, P.A. Extended-spectrum beta-lactamases in the 21st Century: Characterization, Epidemiology, and Detection of this Important Resistance Threat. *Clin. Microbiol. Rev.* **2001**, *14*, 933–951. [CrossRef] [PubMed]
3. Bonnet, R. Growing Group of Extended-spectrum beta-lactamases: The CTX-M Enzymes. *Antimicrob. Agents. Chemother.* **2004**, *48*, 1–14. [CrossRef]
4. Rossolini, G.M.; D’Andrea, M.M.; Mugnaioli, C. The Spread of CTX-M-type Extended-spectrum β -lactamases. *Clin. Microbiol. Infect.* **2008**, *14*, 33–41. [CrossRef] [PubMed]
5. Jacoby, G.A. AmpC β -lactamases. *Clin. Microbiol. Rev.* **2009**, *22*, 161–182. [CrossRef] [PubMed]
6. Poirel, L.; Pitout, J.D.; Nordmann, P. Carbapenemases: Molecular Diversity and Clinical Consequences. *Future Microbiol.* **2007**, *2*, 501–512. [CrossRef]
7. Cantón, R.; Akóva, M.; Carmeli, Y.; Giske, C.G.; Glupczynski, Y.; Gniadkowski, M.; Livermore, D.M.; Miriagou, V.; Naas, T.; Rossolini, G.M.; et al. European Network on Carbapenemases. Rapid Evolution and Spread of Carbapenemases among *Enterobacteriaceae* in Europe. *Clin. Microbiol. Infect.* **2012**, *18*, 413–431. [CrossRef]
8. European Committee for Antimicrobial Susceptibility Testing. Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 15.00. Valid from 2025-01-01. Available online: <http://www.eucast.org> (accessed on 1 October 2025).

9. M100-S22; Performance Standards for Antimicrobial Susceptibility Testing, 35th ed. Approved Standard Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2025.
10. Magiorakos, A.P.; Srinivasan, A.; Carey, R.B.; Carmeli, Y.; Falagas, M.E.; Giske, C.G.; Harbarth, S.; Hindler, J.F.; Kahlmeter, G.; Olsson-Liljequist, B.; et al. Multidrug-resistant, Extensively Drug-resistant and Pandrug-resistant Bacteria: An International Expert Proposal for Interim Standard Definitions for Acquired Resistance. *Clin. Microbiol. Infect.* **2002**, *18*, 268–281. [\[CrossRef\]](#)
11. Polsfuss, S.; Bloemberg, G.V.; Giger, J.; Meyer, V.; Böttger, E.C.; Hombach, M. Practical Approach for Reliable Detection of AmpC β -lactamase-producing *Enterobacteriaceae*. *J. Clin. Microbiol.* **2011**, *49*, 2798–2803. [\[CrossRef\]](#)
12. Lee, K.; Lim, Y.S.; Yong, D.; Yum, J.H.; Chong, Y. Evaluation of the Hodge Test and the Imipenem-EDTA-double-disk Synergy Test for Differentiating Metallo- β -lactamase-producing Isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J. Clin. Microbiol.* **2003**, *41*, 4623–4629. [\[CrossRef\]](#)
13. M100; Performance Standards for Antimicrobial Susceptibility Testing, 31st ed. Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2021.
14. MacDonald, J.W.; Chibabhai, V. Evaluation of the RESIST-4 O.K.N.V immunochromatographic Lateral Flow Assay for the Rapid Detection of OXA-48, KPC, NDM and VIM Carbapenemases from Cultured Isolates. *Access Microbiol.* **2019**, *1*, e000031. [\[CrossRef\]](#)
15. Elwell, L.P.; Falkow, S. The Characterization of R Plasmids and the Detection of Plasmid-specified Genes. In *Antibiotics in Laboratory Medicine*, 2nd ed.; Lorian, V., Ed.; Williams and Wilkins: Baltimore, MD, USA, 1986; pp. 683–721.
16. Arlet, G.; Brami, G.; Decre, D.; Flippo, A.; Gaillot, O.; Lagrange, P.H.; Philippon, A. Molecular Characterization by PCR Restriction Fragment Polymorphism of TEM β -lactamases. *FEMS Microbiol. Lett.* **1995**, *134*, 203–208. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Nüesch-Inderbilen, M.T.; Hächler, H.; Kayser, F.H. Detection of Genes Coding for Extended-spectrum SHV β -lactamases in Clinical Isolates by a Molecular Genetic Method, and Comparison with the E test. *Eur. J. Clin. Microbiol. Infect. Dis.* **1996**, *15*, 398–402. [\[CrossRef\]](#)
18. Woodford, N.; Ward, M.E.; Kaufmann, M.E.; Turton, J.; Fagan, E.J.; James, D.; Johnson, A.P.; Pike, R.; Warner, M.; Cheasty, T.; et al. Community and Hospital Spread of *Escherichia coli* Producing CTX-M Extended-spectrum β -lactamases in the UK. *J. Antimicrob. Chemother.* **2004**, *54*, 735–743. [\[CrossRef\]](#)
19. Pagani, L.; Migliavacca, R.; Pallecchi, L.; Matti, C.; Giacobone, E.; Amicosante, G.; Romero, E.; Rossolini, G.M. Emerging extended-spectrum beta-lactamases in *Proteus mirabilis*. *J. Clin. Microbiol.* **2002**, *40*, 1549–1552. [\[CrossRef\]](#)
20. Robicsek, A.; Jacoby, G.A.; Hooper, D.C. The Worldwide Emergence of Plasmid-mediated Quinolone Resistance. *Lancet Infect. Dis.* **2006**, *6*, 629–640. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Woodford, N.; Fagan, E.J.; Ellington, M.J. Multiplex PCR for Rapid Detection of Genes Encoding CTX-M Extended-spectrum (β)-lactamases. *J. Antimicrob. Chemother.* **2006**, *5*, 154–155. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Perez-Perez, F.J.; Hanson, N.D. Detection of Plasmid-mediated AmpC β -lactamase Genes in Clinical Isolates by Using Multiplex PCR. *J. Clin. Microbiol.* **2002**, *40*, 2153–2162. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Poirel, L.; Walsh, T.R.; Cuveiller, V.; Nordman, P. Multiplex PCR for Detection of Acquired Carbapenemases Genes. *Diagn. Microbiol. Infect. Dis.* **2011**, *70*, 119–123. [\[CrossRef\]](#)
24. Saladin, M.; Cao, V.T.B.; Lambert, T.; Donay, J.L.; Hermann, J.; Ould-Hocine, L. Diversity of CTX-M β -lactamases and Their Promoter Regions from *Enterobacteriaceae* Isolated in Three Parisian Hospitals. *FEMS Microbiol. Lett.* **2002**, *209*, 161–168. [\[CrossRef\]](#)
25. Giani, T.; Conte, V.; Di Pilato, V.; Aschbacher, R.; Weber, C.; Larcher, C.; Rossolini, G.M. *Escherichia coli* from Italy Producing OXA-48 Carbapenemase Encoded by a Novel Tn1999 Transposon Derivative. *Antimicrob. Agents. Chemother.* **2012**, *56*, 2211–2213. [\[CrossRef\]](#)
26. Carattoli, A.; Bertini, A.; Villa, L.; Falbo, V.; Hopkins, K.L.; Threlfall, E.J. Identification of Plasmids by PCR-based Replicon Typing. *J. Microbiol. Methods.* **2005**, *63*, 219–228. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Carattoli, A.; Seiffert, S.N.; Schwendener, S.; Perreten, V.; Endimiani, A. Differentiation of IncL and IncM Plasmids Associated with the Spread of Clinically Relevant Antimicrobial Resistance. *PLoS ONE* **2015**, *10*, e0123063. [\[CrossRef\]](#)
28. Nirca, V.; Fuchs, F.; Burgwinkel, T.; Del Pino, R.A.; Zaharcenco, E.; Hagen, R.M.; Poppert, S.; Frickmann, H.; Higgins, P.G. Cross-Sectional Assessment on Carbapenem-Resistant Gram-Negative Bacteria Isolated from Patients in Moldova. *Microorganisms* **2025**, *14*, 421. [\[CrossRef\]](#)
29. Jünemann, S.; Sedlazeck, F.J.; Prior, K.; Albersmeier, A.; John, U.; Kalinowski, J.; Mellmann, A.; Goesmann, A.; von Haeseler, A.; Stoye, J.; et al. MBioSEQ Ridom Typer version 11.1.0 (EULA) Updating Benchtop Sequencing Performance Comparison. *Nat. Biotechnol.* **2013**, *31*, 294–296. [\[CrossRef\]](#)
30. Jolley, K.A.; Bray, J.E.; Maiden, M.C.J. Open-access Bacterial Population Genomics: BIGSdb Software, the PubMLST.org Website and Their Applications. *Wellcome Open Res.* **2018**, *3*, 124. [\[CrossRef\]](#)
31. Linkevicius, M.; Witteveen, S.; Buzea, M.; Flonta, M.; Indreas, M.; Nica, M.; Székely, E.; Tălăpan, D.; Svartström, O.; Alm, E.; et al. Genomic Surveillance Detects Interregional Spread of New Delhi Metallo-beta-lactamase-1-producing *Providencia stuartii* in Hospitals, Romania, December 2021 to September 2023. *Euro. Surveill.* **2024**, *29*, 2400587. [\[CrossRef\]](#) [\[PubMed\]](#)

32. Molnár, S.; Flonta, M.M.; Almaş, A.; Buzea, M.; Licker, M.; Rus, M.; Földes, A.; Székely, E. Dissemination of NDM-1 Carbapenemase-producer *Providencia stuartii* Strains in Romanian Hospitals: A Multicentre Study. *J. Hosp. Infect.* **2019**, *103*, 165–169. [CrossRef] [PubMed]
33. Sabtcheva, S.; Stoikov, I.; Ivanov, I.N.; Donchev, D.; Lesseva, M.; Georgieva, S.; Teneva, D.; Dobрева, E.; Christova, I. Genomic Characterization of Carbapenemase-Producing *Enterobacter hormaechei*, *Serratia marcescens*, *Citrobacter freundii*, *Providencia stuartii*, and *Morganella morganii* Clinical Isolates from Bulgaria. *Antibiotics* **2024**, *13*, 455. [CrossRef]
34. Manageiro, V.; Sampaio, D.A.; Pereira, P.; Rodrigues, P.; Vieira, L.; Palos, C.; Caniça, M. Draft Genome Sequence of the First NDM-1-Producing *Providencia stuartii* Strain Isolated in Portugal. *Genome. Announc.* **2015**, *3*, e01077-15. [CrossRef]
35. McGann, P.; Hang, J.; Clifford, R.J.; Yang, Y.; Kwak, Y.I.; Kuschner, R.A.; Lesho, E.P.; Waterman, P.E. Complete Sequence of a Novel 178-kilobase Plasmid Carrying blaNDM-1 in a *Providencia stuartii* Strain Isolated in Afghanistan. *Antimicrob. Agents. Chemother.* **2012**, *56*, 1673–1679. [CrossRef]
36. Gefen-Halevi, S.; Hindiyeh, M.Y.; Ben-David, D.; Smollan, G.; Gal-Mor, O.; Azar, R.; Castanheira, M.; Belausov, N.; Rahav, G.; Tal, I.; et al. Isolation of Genetically Unrelated bla(NDM-1)-positive *Providencia rettgeri* Strains in Israel. *J. Clin. Microbiol.* **2013**, *51*, 1642–1643. [CrossRef]
37. Oikonomou, O.; Liakopoulos, A.; Phee, L.M.; Betts, J.; Mevius, D.; Wareham, D.W. *Providencia stuartii* Isolates from Greece: Co-Carriage of Cepha-losporin blaSHV-5 blaVEB-1, Carbapenem blaVIM-1, and Aminoglycoside rmtB Resistance Determinants by a Multidrug-Resistant Outbreak Clone. *Microb. Drug. Resist.* **2016**, *225*, 379–386. [CrossRef]
38. Guidone, G.H.M.; Cardozo, J.G.; Silva, L.C.; Sanches, M.S.; Galhardi, L.C.F.; Kobayashi, R.K.T.; Vespero, E.C.; Rocha, S.P.D. Epidemiology and Characterization of *Providencia stuartii* Isolated from Hospitalized Patients in Southern Brazil: A Possible Emerging Pathogen. *Access. Microbiol.* **2023**, *5*, 000652.v4. [CrossRef]
39. Bocanegra-Ibarias, P.; Duran-Bedolla, J.; Silva-Sánchez, J.; Garza-Ramos, U.; Sánchez-Pérez, A.; Garza-González, E.; Morfín-Otero, R.; Barrios-Camacho, H. Identification of *Providencia* spp. Clinical Isolates Co-producing Carbapenemases IMP-27, OXA-24, and OXA-58 in Mexico. *Diagn. Microbiol. Infect. Dis.* **2024**, *109*, 116246. [CrossRef] [PubMed]
40. Otlu, B.; Yakupoğulları, Y.; Gürsoy, N.C.; Duman, Y.; Bayındır, Y.; Tekerekoğlu, M.S.; Ersoy, Y. *Providencia rettgeri* de OXA-48 ve NDM-1 Karbapenemaz Genlerinin Birlikte üretimi: İlk bildirim Co-production of OXA-48 and NDM-1 Carbapenemases in *Providencia rettgeri*: The First Report. *Mikrobiyol. Bul.* **2018**, *52*, 300–307. (In Turkish) [CrossRef] [PubMed]
41. Croatian Academy of Medical Sciences. *Antibiotic Resistance in Croatia*; Croatian Academy of Medical Sciences: Zagreb, Croatia, 2023; p. 64.
42. EUCAST. Intrinsic Resistance and Unusual Phenotype. Available online: <https://www.eucast.org/news-detail/eucast-on-intrinsic-resistance-and-unusual-phenotypes-updated/> (accessed on 25 October 2025).
43. Pasteran, F.; Gonzalez, L.J.; Albornoz, E.; Bahr, G.; Vila, A.J.; Corso, A. Triton Hodge Test: Improved Protocol for Modified Hodge Test for Enhanced Detection of NDM and Other Carbapenemase Producers. *J. Clin. Microbiol.* **2016**, *54*, 640–649. [CrossRef] [PubMed]
44. Barl, P.; Bedenić, B.; Sardelić, S.; Uzunović, S.; Vraneš, J.; Plečko, V. Spread of CTX-M-15 Positive *Providencia* spp. Causing Urinary Tract Infections at the University Hospital Split in Croatia. *Med. Glas.* **2012**, *9*, 317–324.
45. Rubic, Z.; Soprek, S.; Jelic, M.; Novak, A.; Goic-Barisic, I.; Radic, M.; Tambic-Andrasevic, A.; Tonkic, M. Molecular Characterization of β -Lactam Resistance and Antimicrobial Susceptibility to Possible Therapeutic Options of AmpC-Producing Multidrug-Resistant *Proteus mirabilis* in a University Hospital of Split, Croatia. *Microb. Drug. Resist.* **2021**, *27*, 162–169. [CrossRef]
46. Shin, S.; Jeong, S.H.; Lee, H.; Hong, J.S.; Park, M.J.; Song, W. Emergence of Multidrug-resistant *Providencia rettgeri* Isolates Co-producing NDM-1 Carbapenemase and PER-1 Extended-spectrum β -lactamase Causing a First Out-break in Korea. *Ann. Clin. Microbiol. Antimicrob.* **2018**, *17*, 20. [CrossRef]
47. Capitani, V.; Arcari, G.; Oliva, A.; Sacco, F.; Menichincheri, G.; Fenske, L.; Polani, R.; Raponi, G.; Antonelli, G.; Carattoli, A. Genome-Based Retrospective Analysis of a *Providencia stuartii* Outbreak in Rome, Italy: Broad Spectrum IncC Plasmids Spread the NDM Carbapenemase within the Hospital. *Antibiotics* **2023**, *12*, 943. [CrossRef] [PubMed]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.