Imipenem Resistance Mediated by $\text{bla}_{\text{OXA-913}}$ Gene in Pseudomonas aeruginosa

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Academic Editors: Michael Calcutt and Anna Psaroulaki

Received: 9 August 2021 Accepted: 28 September 2021 Published: 29 September 2021

Citation: Moon, D.-C.; Mechesso, A.F.; Kang, H.-Y.; Kim, S.-J.; Choi, J.-H.; Song, H.-J.; Yoon, S.-S.; Lim, S.-K. Imipenem Resistance Mediated by $\text{bla}_{\text{OXA-913}}$ Gene in Pseudomonas aeruginosa. Antibiotics 2021, 10, 1188. https://doi.org/10.3390/antibiotics10101188

Abstract: Treatment of infectious diseases caused by carbapenem-resistant Pseudomonas aeruginosa is becoming a greater challenge. This study aimed to identify the imipenem resistance mechanism in P. aeruginosa isolated from a dog. Minimum Inhibitory Concentration (MIC) was determined by the broth microdilution method according to the Clinical and Laboratory Standards Institute recommendations. We performed polymerase chain reaction and whole-genome sequencing to detect carbapenem resistance genes. Genomic DNA of P. aeruginosa K19PSE24 was sequenced via the combined analysis of 20-kb PacBio SMRTbell and PacBio RS II. Peptide-Peptide Nucleic Acid conjugates (P-PNAs) targeting the translation initiation region of $\text{bla}_{\text{OXA-913}}$ were synthesized. The isolate (K19PSE24) was resistant to imipenem and piperacillin/tazobactam yet was susceptible to most of the tested antimicrobials. Whole-genome sequencing revealed that the K19PSE24 genome comprised a single contig amounting to 6,815,777 base pairs, with 65 tRNA and 12 rRNA genes. K19PSE24 belonged to sequence type 313 and carried the genes $\text{oxyA}$, $\text{fetA}$, $\text{catB7}$, $\text{crpP}$, and $\text{bla}_{\text{OXA-913}}$ (an allele deposited in GenBank but not described in the literature). K19PSE24 also carried genes encoding for virulence factors (exoenzyme T, exotoxin A, and elastase B) that are associated with adhesion, invasion, and tissue lysis. Nevertheless, we did not detect any of the previously reported carbapenem resistance genes. This is the first report of the $\text{bla}_{\text{OXA-913}}$ gene in imipenem-resistant P. aeruginosa in the literature. Notably, no viable colonies were found after co-treatment with imipenem (2 µg/mL) and either of the P-PNAs (12.5 µM or 25 µM). The imipenem resistance in K19PSE24 was primarily due to $\text{bla}_{\text{OXA-913}}$ gene carriage.

Keywords: $\text{bla}_{\text{OXA-913}}$; imipenem; P. aeruginosa; resistance mechanism

1. Introduction

Pseudomonas aeruginosa is one of the most problematic opportunistic human pathogens and is particularly evident in cases of hospital-acquired pneumonia in immunocompromised patients. It can be transmitted from humans to companion animals or vice versa [1,2]. Carbapenems are currently the antibiotics of choice for the treatment of infections caused by multidrug-resistant pathogens. Carbapenems bind to penicillin-binding proteins and inactivate an inhibitor of autolytic enzymes within the cell wall which leads to the death of the bacteria [3]. Currently, different kinds of carbapenems are used in clinical practice as antipseudomonal agents, such as doripenem, imipenem, and meropenem [4]. Among these, meropenem is commonly used to treat bacteremia, sepsis, and infections caused by resistant bacteria in dogs and cats [5,6]. Adverse events are relatively rare with carbapenems and are mostly minor [7].

The global emergence of carbapenemase-producing bacteria is an alarming signal, potentially leading to ever-increasingly restricted therapeutic choices [8]. Carbapenemases are primarily classified into three classes of β-lactamases, the ambler classes A, B, and
D β-lactamases. Class D β-lactamases, also known as oxacillinas (OXA), have become the most common type of acquired carbapenemases [9]. They are characterized by rapid mutation and an expanded spectrum of activity [10]. Carbapenem-hydrolyzing class D β-lactamases have been observed in Enterobacteriales with OXA-48-like; in Acinetobacter baumannii with OXA-23-like, OXA-40-like, OXA-58-like, and OXA-143-like; and in P. aeruginosa with OXA-40-like, OXA-48-like, OXA-181-like, and OXA-198-like [10–14]. Recent emerging mechanisms of carbapenem resistance accumulate through the spread of carbapenem-destroying-β-lactamases and leave a narrowed range of therapeutic options. In this study, we aimed to determine the imipenem resistance mechanism in a clinical isolate of P. aeruginosa recovered from a dog with pyoderma.

2. Results and Discussion

PacBio SMART analysis demonstrated that the genome of P. aeruginosa (K19PSE24) comprised 6,815,777 base pairs with total coverage of 151.0× and 66.11% GC content. Abundant categories in the Cluster of Orthologous Groups (COG) distribution (>5% of the total COG-matched counts) include amino acid and inorganic ion transport and metabolism. We also identified virulence factors that are associated with focal adhesion, phagocytosis, and subsequent dissemination of P. aeruginosa (exoenzyme T, exoT), tissue lysis and invasion (exotoxin A, exoA), and acute infection (elastase B, lasB) [15,16]. Multilocus sequence typing demonstrated that the strain belonged to sequence type 313, a type already reported in patients from different countries, including South Korea [17–19].

In this study, P. aeruginosa (K19PSE24) was resistant to imipenem (MIC > 8 µg/mL) and piperacillin/tazobactam (128/4 µg/mL), while it was susceptible to most of the tested antimicrobials, including meropenem and ceftazidime (Table S1). K19PSE24’s chromosome possessed genes encoding resistance to aminoglycosides (aph(3)-IIb), β-lactams (blaOXA-913 and blaPAO), fosfomycin (fosA), phenicols (catB7), and quinolones (crpP). We did not, however, detect any of the previously reported carbapenem resistance-encoding genes using PCR and a whole-genome sequencing assay. Acquisition of multiple imported and chromosomally encoded resistance mechanisms and/or a single mutational event contribute to multidrug resistance in P. aeruginosa [20]. Fosfomycin, one of the oldest antimicrobials, has now been revisited for its possible effectiveness against multidrug-resistant strains, including P. aeruginosa [21]. Thus, further investigations may be needed on the contribution of the fosA gene on the susceptibility of P. aeruginosa to fosfomycin. Several studies have demonstrated the prevalence of the blaPAO gene, a chromosomally-encoded cephalosporinase gene, in P. aeruginosa [22–24]. Madaha et al. [25] reported that blaPAO-carrying P. aeruginosa isolates were sensitive to carbapenems especially imipenem. The blaOXA-913 gene has also been reported in P. aeruginosa in Switzerland (GenBank: NG-068184.1); however, the finding has not been described in the literature. Here, we report for the first time the blaOXA-913 gene in imipenem-resistant P. aeruginosa isolated from a dog with pyoderma in South Korea. We found low similarity (36.5%) between the amino acid sequences of the blaOXA-913 gene detected in this study and the blaOXA-48-like gene in K. pneumoniae (GenBank accession number CP024838.1). In addition, except for a single amino acid substitution (Asn99Lys), blaOXA-913 in this study was highly similar (99.6%) to the blaOXA-488 gene identified in P. aeruginosa (NG-049768.1). The chromosomal regions of the blaOXA-913 gene had a substantial sequence homology (>98% sequence identity) compared to those of previously reported OXA-50 family class D genes in P. aeruginosa from different sources (Figure 1). Furthermore, we noted co-linearity (identity ≈ 99%) between the nucleotide sequences of our strain (CP053687) and those of the OXA-50 family gene-carrying P. aeruginosa strains described in Figure 1, as well as that of P. aeruginosa PAO1 (NC_002516.2). The detection of a new carbapenem resistance gene (blaOXA-913) in a virulent strain of P. aeruginosa is a great concern, since it significantly restricts the therapeutic options for patients.
Figure 1. Comparative analysis of chromosomal regions (1 Mb) of the \textit{bla}_{OXA-913} gene and OXA-50 family class D genes. ANI analysis was performed using the ANI-blast method implemented in PYANI (v.0.2.10) and the tree was generated based on the ANI values. The horizontal lines represent the 95% threshold value. The scale bar represents sequence divergence, i.e., the percentage of nucleotide substitution rate over the length of the genome.

PNA is an artificially synthesized DNA mimic that forms a stable complex with DNA and RNA molecules in a sequence-dependent manner. Previous studies have shown the target-specific gene silencing and/or growth inhibitory activities of P-PNAs in Gram-positive and Gram-negative bacteria [26,27]. Ray and Norden [28] revealed that P-PNAs can form a stable link with DNA and interfere with replication or transcription of the target genes. In this study, the P-PNAs were designed to target the translation initiation region of \textit{bla}_{OXA-913}. Growth inhibition was not observed when \textit{P. aeruginosa} (K19PSE24) was treated with either the P-PNAs (0.4–25 \(\mu\)M/mL) or imipenem (2 \(\mu\)g/mL). However, no viable colonies were recovered after co-treatment with imipenem (2 \(\mu\)g/mL) and either 12.5 \(\mu\)M/mL or 25 \(\mu\)M/mL of the P-PNAs. Therefore, the imipenem resistance in K19PSE24 was primarily due to \textit{bla}_{OXA-913} gene carriage.

3. Materials and Methods

3.1. Isolation and Identification of \textit{P. aeruginosa}

An isolate of \textit{P. aeruginosa} was obtained from a skin scraping specimen of a dog with pyoderma in 2019. Isolation and identification of \textit{P. aeruginosa} were performed using a CHROMagar\textsuperscript{TM} \textit{Pseudomonas} agar plate (CHROMagar, Becton Dickinson, Sparks, MD, USA). The isolate was then confirmed by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF, bioMérieux, Marcy L’Etoile, France).

3.2. Antimicrobial Susceptibility Testing

Testing for antimicrobial susceptibility was performed by the broth microdilution method using the COMPGN1F Sensititre panel (Trek Diagnostic Systems, Cleveland, OH, USA), according to the manufacturer’s instruction. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) breakpoints. \textit{P. aeruginosa} ATCC27853 was used as a reference strain.
3.3. Polymerase Chain Reaction (PCR) and Whole-Genome Sequencing

A PCR assay was performed to detect the most frequently reported carbapenem resistance genes in South Korea (\textit{bla}\textsubscript{IMP}, \textit{bla}\textsubscript{VIM}, \textit{bla}\textsubscript{OXA-48-like}, \textit{bla}\textsubscript{NDM}, and \textit{bla}\textsubscript{KPC}) (Table S2) \[29,30\]. Whole-genome sequencing was performed using PacBio RS II (Pacific Biosciences, Menlo Park, CA, USA), as previously described \[31\]. Antimicrobial resistance genes were analyzed by the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/; accessed on 18 February 2021). The sequence type (ST) of \textit{P. aeruginosa} (GenBank accession number CP053687.1) was inferred from \textit{Pseudomonas} housekeeping genes using the Multilocus Sequence Typing Application 1.8 \[32\]. In addition, the sequence of the chromosomal regions of the \textit{bla}\textsubscript{OXA-913} gene in this study (CP053687) was compared with those of previously reported OXA-50 family class D genes. Briefly, the nucleotide sequences of complete genomes of the OXA-50 family class D gene-carrying \textit{P. aeruginosa} were downloaded from the GenBank nucleotide database, sequences were trimmed and chromosomal regions (1Mb) containing \textit{bla}\textsubscript{OXA} genes were prepared. The average nucleotide identity (ANI) values were calculated with pairwise genome alignment of sequences by using the ANI-blast method implemented in PYANI (v.0.2.10) \[33\], and the phylogenetic tree was reconstructed based on the ANI values. In addition, the nucleotide sequence of our strain was compared with those of OXA-50 family class D gene-carrying strains used in ANI analysis, as well as that of \textit{P. aeruginosa} PAO1 (NC_002516.2).

3.4. Peptide-Peptide Nucleic Acid Conjugation

We used artificially synthesized peptide-peptide nucleic acid conjugates (P-PNAs) ((KFF)\textsubscript{3K-L-ATGCGCCCTCTCCTTTACCAG and (KFF)\textsubscript{3K-L-CGAGCCATGCGCCCTCTCCT}, 5’ to 3’ sequence) to silence the \textit{bla}\textsubscript{OXA-913} gene and confirm the gene associated with imipenem resistance. Briefly, the \textit{bla}\textsubscript{OXA-913}-specific oligonucleotides were searched in the genome sequence of \textit{P. aeruginosa} (CP053687.1). The resulting P-PNA oligomers for \textit{bla}\textsubscript{OXA-913} were designed to bind to its translation initiation region, which overlapped the ATG start codon and the ribosome-binding Shine-Dalgarno sequences (CGAGCC). PNA synthesis, purification, and conjugation with (KFF)\textsubscript{3K-L} bacterial penetration peptide were performed at PANAGENE (Daejeon, South Korea). \textit{P. aeruginosa} (K19PSE24 and ATCC 27853, 5 \times 10^{4} CFU/mL) were incubated with imipenem (2 \mu g/mL) alone or in combination with different concentrations of P-PNAs (0.4, 0.8, 1.6, 3.12, 6.25, 12.5, and 25 \mu M) in 100 \mu L of Muller-Hinton broth at 37 °C for four hours. Then, 50 \mu L was removed and spread-plated on Muller-Hinton agar plates, and the CFUs were determined after incubation at 37 °C for 18 h.

4. Conclusion

The detection of the \textit{bla}\textsubscript{OXA-913} gene in \textit{P. aeruginosa} is an alarming emerging threat. This study highlights the need for continuous screening of companion animal isolates, given that a novel imipenem resistance gene was detected in an isolate recovered from a dog.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/antibiotics10101188/s1: Table S1: Antimicrobial susceptibility profiles of \textit{P. aeruginosa} K19PSE24; Table S2: Lists of primer used in the detection of carbapenem resistance genes in \textit{P. aeruginosa}.

Author Contributions: Conceptualization, S.-K.L. and D.-C.M.; methodology, D.-C.M., H.-Y.K., and S.-S.Y.; validation, A.F.M., S.-J.K., and J.-H.C.; formal analysis, J.-H.C. and H.-J.S.; investigation, D.-C.M., H.-Y.K., H.-J.S., J.-H.C., A.F.M., and S.-J.K.; data curation, D.-C.M. and S.-S.Y.; writing—original draft preparation, A.F.M. and D.-C.M.; writing—review and editing, A.F.M., S.-S.Y., D.-C.M., and S.-K.L.; supervision, S.-S.Y. and S.-K.L.; project administration, D.-C.M. and H.-Y.K.; funding acquisition; S.-K.L. and D.-C.M. All authors have read and agreed to the published version of the manuscript.
Funding: This research was funded by the Animal and Plant Quarantine Agency, Ministry of Agriculture, Food, and Rural Affairs, South Korea, grant B-1543081-2021-21-01.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The nucleotide sequence of the K19PSE24 genome has been submitted to the GenBank nucleotide sequence database and assigned accession number CP053687.1.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Fernandes, M.; Sellera, F.; Moura, Q.; Carvalho, M.P.; Rosato, P.N.; Cerdeira, L.; Lincopan, N. Zoonanthroponotic Transmission of Drug-Resistant Pseudomonas aeruginosa, Brazil. Emerg. Infect. Dis. 2018, 24, 1160–1162. [CrossRef] [PubMed]

2. Ramieze-Estrada, S.; Borgatta, B.; Rello, J. Pseudomonas aeruginosa Ventilator-associated Pneumonia Management. Infect. Drug Resist. 2016, 9, 7–18. [CrossRef] [PubMed]

3. Nicoletti, G.; Russo, G.; Bonfiglio, G. Recent developments in carbapenems. Expert Opin. Investig. Drugs 2002, 11, 529–544. [CrossRef] [PubMed]

4. Watkins, R.R.; Bonomo, R.A. Increasing prevalence of carbapenem-resistant Enterobacteriaceae and strategies to avert a looming crisis. Expert Rev. Anti-Infect. Ther. 2013, 11, 543–545. [CrossRef] [PubMed]

5. Byun, S.Y.; Jeong, J.W.; Choi, J.H.; Lee, K.P.; Youn, H.Y.; Maeng, H.I.; Song, K.H.; Koo, T.S.; Seeo, K.W. Pharmacokinetic study of meropenem in healthy beagle dogs receiving intermittent hemodialysis. J. Vet. Pharmacol. Ther. 2016, 39, 560–565. [CrossRef] [PubMed]

6. Papich, M.G. Antibiotic Treatment of Resistant Infections in Small Animals. Vet. Clin. North Am. Small Anim. Pr. 2013, 43, 1091–1107. [CrossRef]

7. Zhanel, G.G.; Wiebe, R.; Dilay, L.; Thomson, K.; Rubinstein, E.; Hoban, D.J.; Noreddin, A.M.; Karlowsky, J.A. Comparative Review of the Carbapenems. Drugs 2007, 67, 1027–1052. [CrossRef]

8. Bonomo, R.A.; Szabo, D. Mechanisms of Multidrug Resistance in Acinetobacter Species and Pseudomonas aeruginosa. Clin. Infect. Dis. 2006, 43, S49–S56. [CrossRef] [PubMed]

9. Meunier, D.; Doumith, M.; Findlay, J.; Mustafa, N.; Mallard, K.; Anson, J.; Panagea, S.; Pike, R.; Wright, L.; Woodford, N.; et al. Carbapenem resistance mediated by blaOXA-181 in Pseudomonas aeruginosa. J. Antimicrob. Chemother. 2016, 71, 2056–2057. [CrossRef] [PubMed]

10. Mathers, A.J.; Hazen, K.C.; Carroll, J.; Yeh, A.J.; Cox, H.L.; Bonomo, R.A.; Sifri, C.D. First Clinical Cases of OXA-48-Producing Carbapenem-Resistant Klebsiella pneumoniae in the United States: The “Menace” Arrives in the New World. J. Clin. Microbiol. 2012, 50, 680–683. [CrossRef]

11. Borah, V.; Saikia, K.K.; Hazarika, N.K. First report on the detection of OXA-48 β-lactamase gene in Escherichia coli and Pseudomonas aeruginosa co-infection isolated from a patient in a Tertiary Care Hospital in Assam. Indian J. Med. Microbiol. 2016, 34, 252–253. [CrossRef]

12. El Garch, F.; Bogaerts, P.; Bebrone, C.; Galleni, M.; Glupczynski, Y. OXA-198, an Acquired Carbapenem-Hydrolyzing Class D β-Lactamase from Pseudomonas aeruginosa. Antimicrob. Agents Chemother. 2011, 55, 4828–4833. [CrossRef]

13. Poirel, L.; Potron, A.; Nordmann, P. OXA-48-like carbapenemases: The phantom menace. J. Antimicrob. Chemother. 2012, 67, 1597–1606. [CrossRef] [PubMed]

14. Sevillano, E.; Gallego, L.; García-Lobo, J. First detection of the OXA-40 carbapenemase in P. aeruginosa isolates, located on a plasmid also found in A. baumannii. Pathol. Biol. 2009, 57, 493–495. [CrossRef] [PubMed]

15. Idris, S.N.A.; Desa, M.N.M.; Aziz, M.N.; Taib, N.M. Antimicrobial susceptibility pattern and distribution of exoU and exoS in clinical isolates of Pseudomonas aeruginosa at a Malaysian hospital. Southeast Asian J. Trop. Med. Public Health 2012, 43, 116–123. [PubMed]

16. Bradbury, R.; Roddam, L.; Merritt, A.; Reid, D.; Champion, A.C. Virulence gene distribution in clinical, nosocomial and environmental isolates of Pseudomonas aeruginosa. J. Med. Microbiol. 2010, 59, 881–890. [CrossRef]

17. Bocharova, Y.; Savinova, T.; Shagin, D.; Shelenkov, A.A.; Mayanskiy, N.A.; Chebotar, I.V. Inactivation of the oprD porin gene by a novel insertion sequence ISPa195 associated with large deletion in a carbapenem-resistant Pseudomonas aeruginosa clinical isolate. J. Glob. Antimicrob. Resist. 2019, 17, 309–311. [CrossRef]

18. Cho, H.H.; Kwon, K.C.; Kim, S.; Koo, S.H. Correlation Between Virulence Genotype and Fluoroquinolone Resistance in Carbapenem-Resistant Pseudomonas aeruginosa. Ann. Lab. Med. 2014, 34, 286–292. [CrossRef]

19. Libisch, B.; Wätine, J.; Balogh, B.; Gaes, M.; Muzslay, M.; Szabó, G.; Füzı, M. Molecular typing indicates an important role for two international clonal complexes in dissemination of VIM-producing Pseudomonas aeruginosa clinical isolates in Hungary. Res. Microbiol. 2008, 159, 162–168. [CrossRef]
20. De Groote, V.N.; Fauvarrt, M.; Kint, C.I.; Verstraeten, N.; Jans, A.; Cornelis, P.; Michiles, J. *Pseudomonas aeruginosa* fosfomycin resistance mechanisms affect non-inherited fluoroquinolone tolerance. *J. Med. Microbiol.* 2011, 60, 329–336. [CrossRef]

21. Lister, P.D.; Wolter, D.J.; Hanson, N.D. Antibacterial-Resistant *Pseudomonas aeruginosa*: Clinical Impact and Complex Regulation of Chromosomally Encoded Resistance Mechanisms. *Clin. Microbiol. Rev.* 2009, 22, 582–610. [CrossRef]

22. Bornin, R.A.; Bogaerts, P.; Girlich, D.; Huang, T.-D.; Dortet, L.; Glupczynski, Y.; Naas, T. Molecular Characterization of OXA-198 Carbapenemase-Producing *Pseudomonas aeruginosa* Clinical Isolates. *Antimicrob. Agents Chemother.* 2018, 62, e02496-17. [CrossRef]

23. Grandjean, T.; Le Guern, R.; Duployez, C.; Faure, K.; Kipnis, E.; Dessein, R. Draft Genome Sequences of Two *Pseudomonas aeruginosa* Multidrug-Resistant Clinical Isolates, PAL0.1 and PAL1.1. *Microbiol. Resour. Announc.* 2018, 7, e00940-18. [CrossRef]

24. Hussain, M.; Suliman, M.; Ahmed, A.; Altayb, H.; Elneima, E. Draft Genome Sequence of a Multidrug-Resistant *Pseudomonas aeruginosa* Strain Isolated from a Patient with a Urinary Tract Infection in Khartoum, Sudan. *Genome Announc.* 2017, 5, e00203-17. [CrossRef] [PubMed]

25. Madaha, E.L.; Mienie, C.; Gonsu, H.K.; Bughe, R.N.; Fonkoua, M.C.; Mbacham, W.F.; Alayande, K.A.; Bezuidenhout, C.C.; Ateba, C.N. Whole-genome sequence of multi-drug resistant *Pseudomonas aeruginosa* strains UY1PSABAL and UY1PSABAL2 isolated from human broncho-alveolar lavage, Yaoundé, Cameroon. *PLoS ONE* 2020, 15, e0238390. [CrossRef] [PubMed]

26. Ghosal, A.; Nielsen, P.E. Potent Antibacterial Antisense Peptide–Peptide Nucleic Acid Conjugates Against *Pseudomonas aeruginosa*. *Nucleic Acid Ther.* 2012, 22, 323–334. [CrossRef] [PubMed]

27. Maekawa, K.; Azuma, M.; Okuno, Y.; Tsukamoto, T.; Nishiguchi, K.; Setsukinai, K.-I.; Maki, H.; Numata, Y.; Takemoto, H.; Rokushima, M. Antisense peptide nucleic acid–peptide conjugates for functional analyses of genes in *Pseudomonas aeruginosa*. *Bioorg. Med. Chem.* 2015, 23, 7234–7239. [CrossRef] [PubMed]

28. Ray, A.; Norden, B. Peptide nucleic acid (PNA): Its medical and biotechnical applications and promise for the future. *FASEB J.* 2000, 14, 1041–1060. [CrossRef] [PubMed]

29. Pragasam, A.K.; Sahni, R.D.; Anandan, S.; Sharma, A.; Gopi, R.; Hadibasha, N.; Gunasekaran, P.; Veeraraghavan, B. A Pilot Study on Carbapenemase Detection: Do We See the Same Level of Agreement as with the CLSI Observations. *J. Clin. Diagn. Res.* 2016, 10, DC09–DC13. [CrossRef] [PubMed]

30. Dallenne, C.; Da Costa, A.; Decré, D.; Favier, C.; Arlet, G. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in *Enterobacteriaceae*. *J. Antimicrob. Chemother.* 2010, 65, 490–495. [CrossRef]

31. Moon, D.; Kim, S.-J.; Mechesso, A.; Kang, H.; Song, H.-J.; Choi, J.-H.; Yoon, S.-S.; Lim, S.-K. Mobile Colistin Resistance Gene *mcr*-1 Detected on an IncI2 Plasmid in *Salmonella Typhimurium* Sequence Type 19 from a Healthy Pig in South Korea. *Microorganisms* 2021, 9, 398. [CrossRef]

32. Larsen, M.V.; Cosentino, S.; Rasmussen, S.; Friis, C.; Hasman, H.; Marvig, R.L.; Jelsbak, L.; Sicheritz-Pontén, T.; Ussery, D.W.; Aarestrup, F.M.; et al. Multilocus Sequence Typing of Total-Genome-Sequenced Bacteria. *J. Clin. Microbiol.* 2012, 50, 1355–1361. [CrossRef] [PubMed]

33. Pritchard, L.; Glover, R.H.; Humphris, S.; Elphinstone, J.G.; Toth, I.K. Genomics and Taxonomy in Diagnostics for Food Security: Soft-rotting Enterobacterial Plant Pathogens. *Anal. Methods* 2016, 8, 12–24. [CrossRef]