Veterinary Microbiology 292 (2024) 110063

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Phenotypic and genotypic characterization of resistance and virulence in *Pseudomonas aeruginosa* isolated from poultry farms in Egypt using whole genome sequencing

Amira M. Rizk [a], Marwa M. Elsayed [a], Ashraf A. Abd El Tawab [a], Fatma I. Elhofy [a], Enas A. Soliman [a], Tamara Kozytska [b], Hanka Brangsch [b], Lisa D. Sprague [b], Heinrich Neubauer [b], Gamal Wareth [a,b,c,*]

[a] Department of Bacteriology, Immunology, and Mycology, Faculty of Veterinary Medicine, Benha University, Toukh 13736, Moshtohor, Egypt
[b] Institute of Bacterial Infections and Zoonoses, Friedrich-Loeffler-Institut, Jena 07743, Germany
[c] Institute of Infectious Diseases and Infection Control, Jena University Hospital, Jena 07747, Germany

ARTICLE INFO

Keywords:
Pseudomonas aeruginosa
Poultry
WGS
Resistomes
Virulomes
Egypt

ABSTRACT

Pseudomonas aeruginosa (*P. aeruginosa*) is an ESKAPE pathogen that can quickly develop resistance to most antibiotics. This bacterium is a zoonotic pathogen that can be found in humans, animals, foods, and environmental samples, making it a One-Health concern. *P. aeruginosa* threatens the poultry industry in Egypt, leading to significant economic losses. However, the investigation of this bacterium using NGS technology is nearly non-existent in Egypt. In this study, 38 isolates obtained from broiler farms of the Delta region were phenotypically investigated, and their genomes were characterized using whole genome sequencing (WGS). The study found that 100% of the isolates were resistant to fosfomycin and harbored the *fos*A gene. They were also resistant to trimethoprim/sulfamethoxazole, although only one isolate harbored the *sul*1 gene. Non-susceptibility (resistant, susceptible with increased dose) of colistin was observed in all isolates. WGS analysis revealed a high level of diversity between isolates, and MLST analysis allocated the 38 *P. aeruginosa* isolates into 11 distinct sequence types. The most predominant sequence type was ST267, found in 13 isolates, followed by ST1395 in 8 isolates. The isolates were susceptible to almost all tested antibiotics carrying only few different antimicrobial resistance (AMR) genes. Various AMR genes that confer resistance mainly to ß-lactam, aminoglycoside, sulfonamide, and phenicol compounds were identified. Additionally, several virulence associated genes were found without any significant differences in number and distribution among isolates. The majority of the virulence genes was identified in almost all isolates. The fact that *P. aeruginosa*, which harbors several AMR and virulence-associated factors, is present in poultry farms is alarming and threatens public health. The misuse of antimicrobial compounds in poultry farms plays a significant role in resistance development. Thus, increasing awareness and implementing strict veterinary regulations to guide the use of veterinary antibiotics is required to reduce health and environmental risks. Further studies from a One-Health perspective using WGS are necessary to trace the potential transmission routes of resistance between animals and humans and clarify resistance mechanisms.

1. Introduction

The major cause of multidrug-resistant (MDR) infections is a group of bacteria known as ESKAPE pathogens. These include *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* spp. These pathogens can escape easily from the bactericidal activity of antimicrobial compounds. They are widely spread in hospitals intensive care units (ICUs), environmental habitats, and also in veterinary ICUs. ESKAPE are notorious for causing high morbidity and mortality in immunosuppressed patients, as well as economic loss (Jadimurthy et al., 2022; Zendri et al., 2023). The ESKAPE pathogens have acquired antimicrobial resistance genes, which has caused a reduction in treatment options for serious infections. This has led to an increase in the burden of disease,

* Corresponding author at: The Institute of Bacterial Infections and Zoonoses, Friedrich-Loeffler-Institut, Jena 07743, Germany.
E-mail address: gamal.wareth@fli.de (G. Wareth).

https://doi.org/10.1016/j.vetmic.2024.110063
Received 30 January 2024; Received in revised form 18 March 2024; Accepted 19 March 2024
Available online 20 March 2024

death rates due to treatment failure, and requires a coordinated global response for antimicrobial resistance surveillance (De Oliveira et al., 2020). *Pseudomonas aeruginosa* (*P. aeruginosa*) is a Gram-negative, aerobic, rod-shaped opportunistic bacterium. This bacterium can develop antibiotic resistance and employs various defense mechanisms making it complex and challenging to treat (Kerr and Snelling, 2009).

In Egypt, *P. aeruginosa* is considered a zoonotic pathogen because it is regularly recovered from samples obtained from humans and animals. MDR *P. aeruginosa* has been isolated from dairy cattle specimens, e.g., from rectal and udder swabs or milk samples, from workers' hand swabs and from environmental samples such as feed, water, and bedding materials (Badawy et al., 2023). Recently, carbapenem-resistant and MDR *P. aeruginosa* strains have been isolated from samples of patients in ICUs and confirmed to be one of the most frequently found Gram-negative bacteria causing fever (Mabrouk et al., 2023; Mostafa et al., 2023). *P. aeruginosa* has been linked to severe illnesses in the Egyptian Baladi sheep and goats (Dapgh et al., 2019) as well as in buffaloes and cattle (Elshafiee et al., 2019). It has also been found in the milk of cows with mastitis (Ameen et al., 2019) and in live fish from private fish farms in the Ismailia governorate (Algammal et al., 2020). Over- and misuse of antibiotic application in veterinary medicine and the food production sectors is believed to play a significant role in resistance development (Caneschi et al., 2023).

Poultry farms are prone to bacterial infections causing severe economic losses (Hassan et al., 2020). Isolation of MDR *P. aeruginosa* has been reported from different avian species on poultry farms in various Egyptian governorates (Marouf et al., 2023), causing septicemia, dehydration and high mortality, particularly in newly hatched chicken (Eraky et al., 2020). Extensively drug-resistant (XDR) *P. aeruginosa* strains have been isolated from broiler chicken farms of the Ismailia governorate (Algammal et al., 2023) by means of classical phenotypic antibiotic sensitivity testing. However, the application of Next Generation Sequence (NGS) technology for monitoring and investigating resistance development in *P. aeruginosa* is rare in Egypt (Baiomy et al., 2023). Numerous PCR protocols have been developed to detect resistance genes in *P. aeruginosa*. However, these conventional methods have certain limitations as they can only identify the expected resistance genes according to the primers used. In contrast, the implementation of high-throughput Whole Genome Sequencing (WGS) with an appropriate pipeline containing bioinformatics tools has enabled fast and comprehensive detection of antibiotic resistance genes in bacterial genomes.

Thus, the current study aimed to compare the phenotype and the genotype of *Pseudomonas* spp. isolated from Egyptian broilers with respect to virulence, resistance genes and genome similarity using whole genome sequence analysis.

2. Materials and methods

2.1. Bacterial isolates and identification

This study has been approved by the ethical committee at the Faculty of Veterinary Medicine, Benha University, under the reference number (BUFVTM35–10–22). All *Pseudomonas* spp. isolates used in the current study originated from poultry farms in the Delta region i.e. Al-Qalyubiyya governorate (n=28), Sharqia (n=3), Al Gharbiyah (n=3) and Wady El Natrun (n=4). Samples were collected from pathological lesions of broiler chickens on broiler farms in 2021. A total of 38 *Pseudomonas* spp. strains were obtained from liver (n=10), intestine (n=8), cloacal washings (n=7), lungs (n=6), gallbladder (n=4), and from the yolk sac (n=3). Sample metadata are shown in supplementary Table S1. The isolates were initially identified by classical bacteriological and biochemical tools, i.e. colony morphology, production of yellowish-green fluorescent pigment, Gram stain, and biochemical tests as previously described (Holt et al., 1994). All isolates were sent to the Institute of Bacterial Infections and Zoonoses (IBIZ, Jena, Germany) for confirmation and typing. According to the Nagoya Protocol, the agreement for receiving and using the Egyptian *Pseudomonas* isolates was obtained from the Egyptian Environmental and Affair Agency under the reference Nr. 00306023010800/6.

Isolates were submitted to Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) to confirm identification using a Microflex LT instrument (Bruker Daltonics, Bremen, Germany) following the MALDI Biotyper manufacturer's recommendation on the log score value of 0–3 for species identification. Score values between 2.300 and 3.000 were considered 'highly probable for species identification', and values between 2.000 and 2.290 were considered 'secure genus identification as previously described (Khater et al., 2021).

The identity of the genus and species of each isolate was confirmed by whole-genome sequencing (WGS) data using Kraken2 (v2.0.7_beta) (Wood et al., 2019), and the first match (largest percentage proportion) was considered for the genus and species identification.

2.2. Antibiotics susceptibility testing (AST)

The minimum inhibitory concentration (MIC) was determined by the broth microdilution method using the automated MICRONAUT-S system (MICRONAUT, MERLIN Diagnostics GmbH, Bornheim-Hersel, Germany) according to the manufacturer's instructions. MICRONAUT-S *Pseudomonas* plates were utilized (Catalog Nr. M/E1–221–040, Merlin, Germany) with Cation-Adjusted Mueller-Hinton Broth (CAMHB) (Bruker Daltonics GmbH & Co. KG, Germany). The MIC determination was performed according to the Clinical and Laboratory Standards Institute (CLSI, 2021) breakpoint recommendations for *P. aeruginosa*. The MICRONAUT-S software automatically classified isolates as susceptible, intermediate, and resistant. The test panel included seven antibiotic groups encompassing 17 antibiotics (Table 1). The standard reference strains, *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, were used for quality control.

2.3. WGS and in-silico detection of AMR and virulence genes

An automated extraction method using the QIAcube instrument (QIAGEN, Germany) was applied to extract DNA from heat-inactivated bacterial biomass. Briefly, one colony was resuspended in 140 µL of ultra-high purity water in a 2 mL reaction tube. The reaction tube was then incubated for 30 minutes at 80 °C. 40 µL of lysozyme solution was added and centrifuged briefly. *E. coli* ATCC 25922 biomass was used as

Table 1
Antibiotics present in the MICRONAUT-S *Pseudomonas* MIC plates used in the current study and their concentration ranges.

Antimicrobial group	Antibiotics	Concentration
Monobactams	Aztreonam (AZT)	1–16 mg/L
Cephalosporins	Cefepime (CEP)	1–8 mg/L
	Ceftazidime (CAZ)	0,25–32 mg/L
	Ceftazidime/Avibactam (CAA)	1/4–8/4 mg/L
	Ceftolozan/Tazobactam (CTA)	1/4–8/4 mg/L
Fluoroquinolones	Ciprofloxacin (CIP)	0,0625–8 mg/L
	Levofloxacin (LEV)	0125–8 mg/L
Aminoglycosides	Gentamicin (GEN)	0,25–32 mg/L
	Tobramycin (TOB)	0,25–32 mg/L
	Amikacin (AMK)	4–32 mg/L
Polymyxins	Colistin (COL)	1–8 mg/L
Phosphonic antibiotics	Fosfomycin (FOS)	16–128 mg/L
Dihydrofolate reductase inhibitor / Sulfonamide antibiotic	Trimethoprim/ Sulfamethoxazole (T/S)	1/19–8/ 152 mg/L
Carbapenems	Imipenem (IMP)	1–8 mg/L
	Meropenem (MER)	0125–16 mg/L
Penicillin	Piperacillin (PIP)	4–32 mg/L
	Piperacillin/Tazobactam (PIT)	1/4–128/4 mg/ L

process control. Next, DNA extraction was performed using the QIAamp DNA Mini QIAcube Kit according to the manufacturer's instructions (QIAGEN, Germany).

The Nextera XT DNA Library Prep Kit (Illumina, Inc. San Diego, California, USA) was used to create sequencing libraries. Paired-end sequencing was performed on an Illumina MiSeq sequencer. The Linux-based pipeline WGSBAC was used to analyze unprocessed sequencing data (v2.2.3, https://gitlab.com/FLI_Bioinfo/WGSBAC, accessed on 28 February 2022) as previously described (Linde et al., 2020; Wareth et al., 2022). FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used for quality check and sequencing coverage of the raw data. The SPAdes-based assembler Shovill was applied for assembly (Bankevich et al., 2012). The sequence classifier Kraken2 was used to identify species and contaminations. For determination of virulence-associated genes, WGSBAC used ABRIcate together with the Virulence Factor Database (Chen et al., 2005) was applied. ABRIcate together with the Comprehensive Antibiotic Resistance Database (CARD) (Jia et al., 2017), ResFinder (Zankari et al., 2012), and NCBI's AMRFinderPlus tool (Feldgarden et al., 2019) were applied to identify the genetic characteristics that contribute to AMR. Based on the assembled genomes, WGSBAC determined the classical Multilocus Sequence Typing (MLST) using the software mlst v2.16.1 (https://github.com/tseemann/mlst) with the species-specific scheme for *P. aeruginosa* from PubMLST as developed by (Curran et al., 2004). To create a phylogenetic tree, core-genome single nucleotide polymorphisms (cgSNPs) were detected using Snippy v4.6.0 (https://github.com/tseemann/snippy), employing the strain *Pseudomonas aeruginosa* PAO1 (GenBank accession number ATCC 27853) as the reference genome. The phylogenetic tree was then reconstructed using RAxML v8.2.12 (Stamatakis, 2014) with the cgSNP alignment and visualized by Microreact (Argimón et al., 2016).

For the comparison of the isolates with previously published Egyptian strains, the NCBI's short read archive (SRA) was browsed for paired-read Illumina data of *P. aeruginosa* isolates from Egypt (accessed on 22nd December 2023). To ensure data quality, the datasets were screened for contamination using ConFindr (Low et al., 2023), and those identified as true *P. aeruginosa* datasets were assembled, as described above. Subsequently, species identity was checked by Kraken2, and MLST sequence types were determined as previously described. The sequences of the downloaded genomes were additionally included in the cgSNP analysis with the existing set of isolates.

2.4. Data availability

All study data are included in the article and supporting information. Raw sequencing data have been submitted to the European Nucleotide Archive (ENA). The project accession number is PRJEB71886.

3. Results

3.1. Bacterial identification, whole genome sequencing (WGS) data and MLST analysis

In the current study, *P. aeruginosa* strains were recovered from the liver, intestine, cloaca, lung, gallbladder, and yolk sac of chicken broilers. MALDI-TOF successfully identified all strains at the genus level, while only 22 isolates were identified at the species level as *P. aeruginosa* (Table S1). Species identity was considered correct when the score value was equal to or more than 2,3. WGS confirmed all isolates as *P. aeruginosa.*

The genome sequencing of 38 *Pseudomonas aeruginosa* isolates yielded an average total of 3372,044 reads per isolate (range 1183,630–5976,046). The isolates' mean coverage was 147-fold (ranging from 53-fold to 248-fold). At the genus level, the first match (largest percentage proportion) for all isolates was always "*Pseudomonas*" with an average of 99.1%. At the species level, the first match for all 38 isolates was always "*Pseudomonas aeruginosa*." Genome assembly yielded a genome size with a minimum of 6187,819 bp and a maximum of 6888,570 bp. The GC content was, on average, 66.35%. The mean N50 of the 38 assembled genomes was 227,795 bp (range 166,496 – 396,442 bp) (Table S1).

MLST analysis allocated the 38 *P. aeruginosa* isolates into 11 distinct sequence types (STs). The predominant sequence types were ST267, represented by 13 isolates from three different governorates, and ST1395 represented by eight isolates from three different governorates, followed by ST641 which was identified only in three isolates from Al Gharbiyah governorate and ST649, with three isolates each. Two strains were assigned to ST274, ST809, ST1228, and ST 1248, and one isolate each to ST319, ST620, and ST1149, respectively (Table S1).

From the 25 available published genomes of Egyptian *P. aeruginosa* datasets (Supplementary Table S2) retrieved from NCBI, only four met all quality parameters and were included in the cgSNP comparison. The clustering generated from the cgSNP alignment matched the MLST ST attribution of the strains, i.e., strains of the same MLST ST showed a maximum of six SNPs and formed distinct clusters (Fig. 1), whereas the cgSNP differences between strains of different STs amounted to at least 14,000 cgSNPs. The foreign strains included in this analysis showed different MLST STs and, accordingly, did not cluster with the strains from this study.

3.2. Phenotyping and WGS-derived AMR determinants and virulence factors of P. aeruginosa isolates

Antibiotic susceptibility testing (AST) of the 38 *Pseudomonas aeruginosa* strains revealed the absence of an MDR pattern. The strains were susceptible to almost all antibiotics tested. However, all 38 isolates (100%) displayed resistance to trimethoprim/sulfamethoxazole (sulfonamides) and fosfomycin (phosphonic antibiotics) and showed non-susceptibility [resistant (n=12) or were susceptible in increased dose (n=26)] to colistin. Non-susceptibility was also seen for levofloxacin (fluoroquinolones) in eight isolates [resistant (n=4) or susceptible in increased dose (n=4)]. However, six isolates displayed resistance to ciprofloxacin of the same antimicrobial group (fluoroquinolones). Susceptibility at increased doses (I) was seen in seven isolates to aztreonam (monobactam) and piperacillin\tazobactam (ß-lactam) and three isolates to imipenem (carbapenem compound). Only one isolate displayed resistance to gentamicin, two isolates to ceftazidime\avibactam, and three to piperacillin. The highest susceptibility was seen for amikacin, cefepime, ceftolozan\tazobactam, and meropenem (Fig. 2).

A wide variety of resistance genes were determined *in silico* in the *P. aeruginosa* isolates, ranging from 64 to 91 genes associated with resistance in several antimicrobial groups (Table S1). Of them, 37 genes were associated with the efflux pump system and were identified in all isolates. Additionally, *cat*B7 conferring resistance to phenicoles, *fos*A conferring resistance to fosfomycin, *bas*S conferring resistance to peptide, *bcr*.1 conferring resistance to bicyclomycin were also identified in all isolates, followed by *arnA* conferring resistance to a peptide which was found in 92% (n=35) of isolates. Thirty-three genes associated with the production of antibiotic-inactivating enzymes such as β-lactamases were identified, of which *bla*PDC variants, a *bla*OXA variant, and *bla*PAO variants were found in all isolates, and the *bla*PDC.374 was the most prominent found in 86.8% (n=33) of isolates. Moreover, four genes that potentially confer resistance to aminoglycosides were identified. Despite all isolates being susceptible to amikacin, they all harbored *aph*(3′)-IIb. One of the isolates harbored the *ant*(2'')-Ia, which confers resistance to gentamicin/kanamycin/tobramycin and was moreover found to be resistant to gentamicin and tobramycin.

A large number of virulence factors ranging from 215 to 239 genes in each isolate were identified in the poultry isolates. Various virulence factors responsible for bacterial adhesion and colonization, biofilm formation and motility, host immune suppression and immune escape, nutritional/metabolic factors, and exotoxins were identified in almost

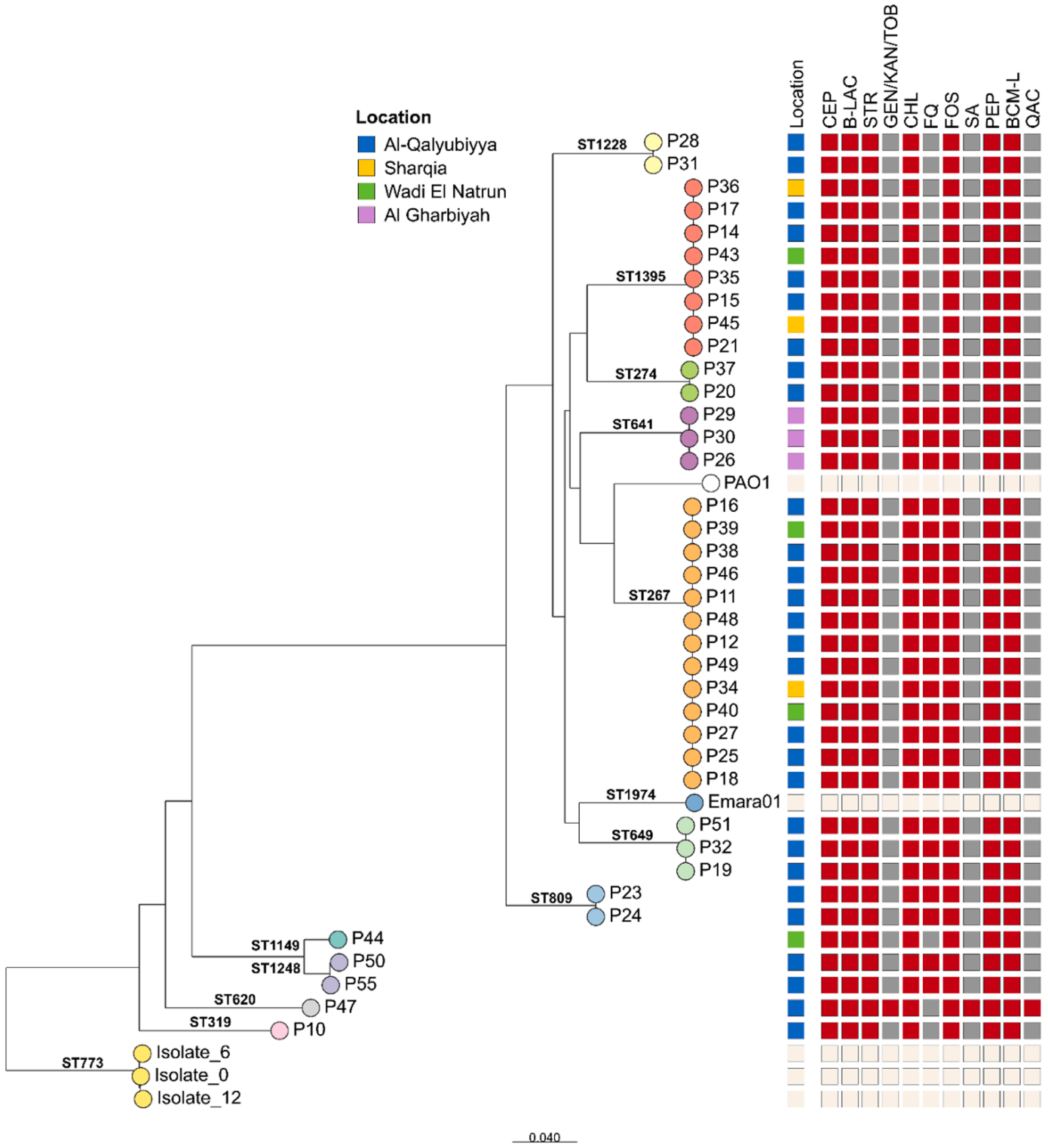

Fig. 1. Maximum likelihood tree generated from cgSNP alignment of *P. aeruginosa* isolates from Egypt. Leaf colors show the MLST sequence type, also indicated at the branches. At the right of the isolate names, the origin of the isolate and the *in-silico* resistance profile to major antibiotic groups is shown (red: resistance genes detected; grey: not detected resistance genes; CEP - cephalosporin, B-LAC - ß-lactams, STR - streptomycin, GEN/KAN/TOB - gentamicin/kanamycin/tobramycin, CHL - chloramphenicol, FQ - fluoroquinolone, FOS - fosfomycin, SA - sulfonamide, PEP - peptides, BCM-L - bicyclomicin-like, QAC - quaternary ammonium compounds). For the foreign data included in the analysis, these data are not shown (crème-colored blocks). The bar at the bottom indicates the number of base substitutions per site.

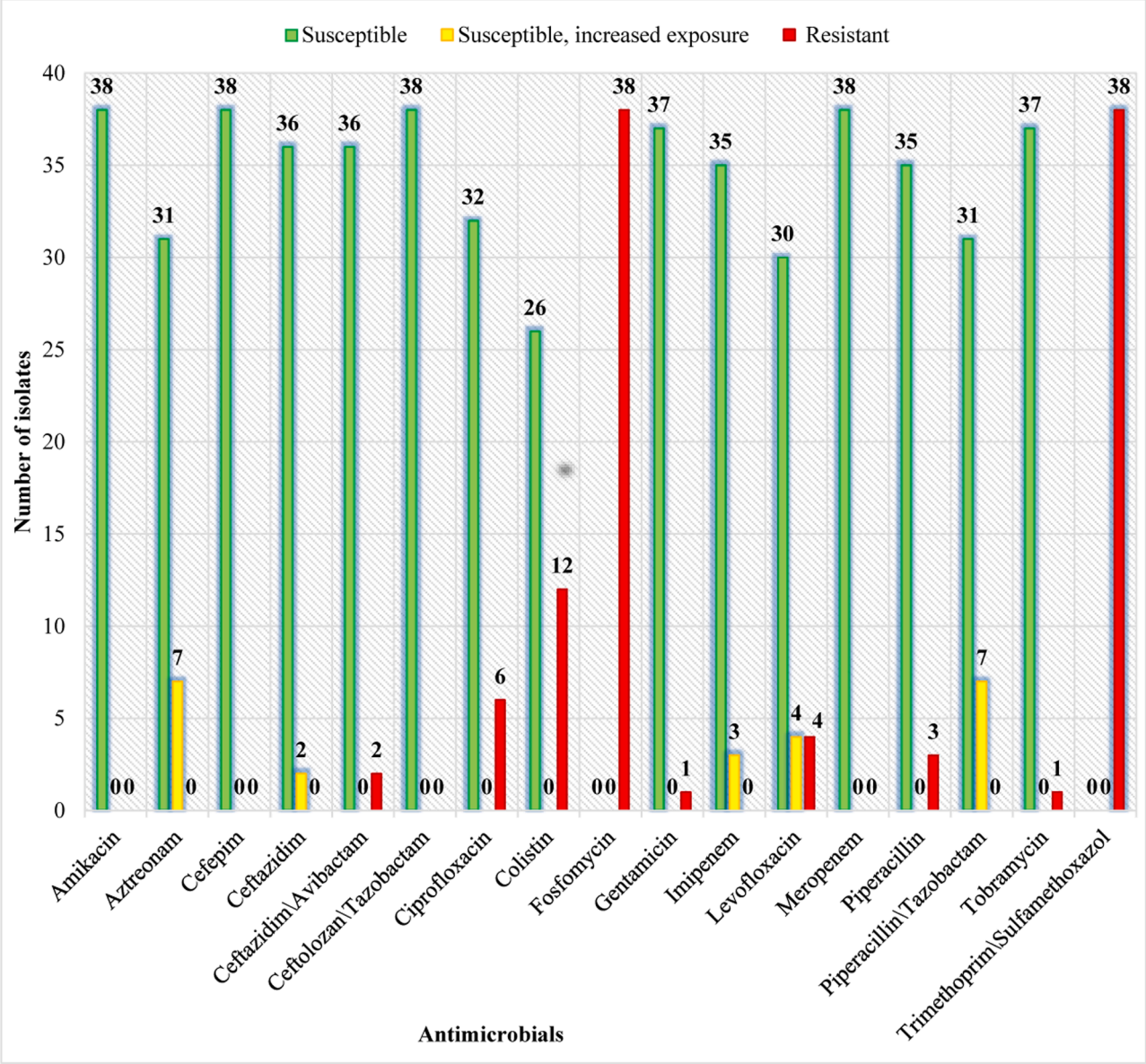

Fig. 2. Susceptible, susceptible at increased exposure and resistant *Pseudomonas aeruginosa* isolates (n=38) and antimicrobials tested are shown.

all isolates. There was no major difference between presence and distribution of virulence genes as nearly all genes were found in all isolates with the exception of two genes (*wzy* and *wzz*) associated with the lipopolysaccharide (LPS) and only found in 13 isolates. Three genes related to pyoverdine (*pvd* I-J) were found in 16 isolates, *pil*C related to Type IV pili was found in 21 isolates, *fli*C, *fli*D and *flg*L related to flagellae were found in 18 isolates. Finally, the *exo*U gene related to TTSS secreted effectors was found only in 5 isolates and *pvd*D related to pyoverdine was found in one isolate only (Table S1).

4. Discussion

Pseudomonas aeruginosa is one of the major global causes of MDR infections. The bacterium can take up DNA from the environment and rapidly develop resistance to multiple classes of antibiotics (Lister et al., 2009). Being a member of ESKAPE pathogens group, it can easily escape the biocidal action of antimicrobial compounds by co-regulating multiple resistance mechanisms, thereby posing a significant therapeutic challenge during treatment (Sathe et al., 2023). *P. aeruginosa* is a serious One Health concern and zoonotic pathogen that has been recently isolated from human and animal samples, as well as from the environment in Egypt (Abd El-Ghany, 2021; Badawy et al., 2023). It is associated with severe infections, particularly of the urinary and respiratory tract, the soft tissue system and sepsis (Pelegrin et al., 2021). In the current study, 38 *P. aeruginosa* strains were isolated from broiler chickens raised on poultry farms of Al-Qalyubiyya, Sharqia, Al Gharbiyah and Wady El Natrun, which have the biggest poultry production of all governorates of the Nile Delta region. None of the isolates displayed MDR patterns. The isolates were obtained from different organs, such as the liver, lung, gall bladder, intestine, and cloaca. It can be assumed that *P. aeruginosa* may regularly cause systematic infection in chickens. This syndrome can be attributed to the variety of virulence factors harbored by *P. aeruginosa* (Edward et al., 2023). The presence of such strains on poultry farms is of particular concern, as they can be transmitted to humans via meat consumption or during handling the meat during slaughter process causing zoonotic infections and may easily spread to other poultry farms.

Pseudomonas aeruginosa is characterized by a relatively large genome (5.5–7 Mbp). Its genetic diversity enables it to grow in different environments. Due to its ability to survive in harsh conditions, it is capable of

producing various virulence factors and antibiotic-resistant determinants, making it resistant to many currently available antibiotics (Lister et al., 2009; Stover et al., 2000). Despite the presence of a wide variety of AMR genes which can cause resistance to at least eight antimicrobial groups, the current set of strains isolated from poultry is still susceptible to the majority of tested antibiotics, except for colistin (polymyxins), trimethoprim\sulfamethoxazole (sulfonamides) and fosfomycin (phosphonic antibiotics). All isolates harbored *fos*A variants conferring resistance to fosfomycin, while only one isolate harbored *sul*1 conferring resistance to sulfonamides. Sulfonamides, fosfomycin, and colistin are the first-line drugs of choice in treating bacterial infections in poultry farms in Egypt. These compounds are cheap and easily available on the market and are often used without consulting veterinarians. Thus, the overuse of these compounds may have resulted in the development of resistance on poultry farms. Monitoring of antimicrobial susceptibility in Staphylococci isolated over four years (2014–2018) from samples of Egyptian poultry farms showed that the bacteria were highly resistant to fosfomycin and trimethoprim\sulfamethoxazole as well (Hamed et al., 2021). As expected, the strains show high sensitivity to cephalosporins i.e., ceftolozan/tazobactam, cefepime, ceftazidime, and ceftazidime/avibactam, and to carbapenems i.e., meropenem and imipenem. Both antimicrobial compounds are not used in veterinary medicine due to their high price and unavailability on the Egyptian markets for veterinary use. Thus, our findings corroborate the normal situation of production.

A wide variety of AMR genes have been found in our isolates ranging from 64 to 91. The detection of 37 efflux pump system genes in all avian *P. aeruginosa* isolates and 33 genes associated with production of antibiotic-inactivating enzymes such as β-lactamases in most of our isolates is not surprising, as *P. aeruginosa* is known for its high level of intrinsic or induced efflux pumps and β-lactamase production (Breidenstein et al., 2011; Pang et al., 2019). One isolate displayed resistance to gentamicin and to bramycin and harbored the *ant*(2'')-Ia conferring resistance to gentamicin/kanamycin/tobramycin. In contrast, all isolates were susceptible to amikacin despite harboring several AMR genes with the potential of conferring resistance to aminoglycosides. The existence of specific genes in the bacterial genome does not necessarily induce function, as some genes may be switched off (Wareth et al., 2017). Also insufficient gene translation or protein expression will not mediate resistance (Yuan-Chuan., 2020). *P. aeruginosa* can produce a variety of virulence factors that help the bacterium to adapt to harsh environmental conditions (Vidaillac and Chotirmall, 2021). In the current study, all isolates harbored many virulence factor genes ranging from 215 to 239 genes. Only few differences were found in the number and distribution of genes among the different isolates, and the majority of genes was present in almost all isolates. Only very few genes were found in less than half of the isolates. The presence of such a diverse collection of virulence determinants is alarming as it may increase pathogenicity, resistance, morbidity, and mortality in patients with *P. aeruginosa* infections (Edward et al., 2023; Liao et al., 2022). Poultry constitutes about 80% of meat production in Egypt and is one of the major sources of environmental pollution with antibiotics (Dahshan et al., 2015). Therefore, increasing awareness and implementing strict veterinary laws to guide the use of veterinary antibiotics are needed to reduce health and environmental risks.

Over the past decades, several studies have been conducted on *P. aeruginosa* in Egypt using samples obtained from patients, animals, slaughterhouses, milk, poultry, and the environment (Abdelatti et al., 2023; Badawy et al., 2023; Dahshan et al., 2015). The majority of previous studies focused on testing the isolates for antibiotic susceptibility using classical tools such as the disc diffusion method and PCR and revealed the presence of multidrug-resistant (MDR) and extensive drug-resistant (XDR) isolates. However, to our knowledge, investigating resistance and virulence-associated genes in *P. aeruginosa* using whole genome sequencing is not regularly applied in veterinary medicine in Egypt, and only one study has been found in human medicine (Baiomy et al., 2023). Only 25 genomes of *P. aeruginosa* recovered from Egyptian patients have been found in the public database NCBI, of which 21 were excluded from our analysis due to low quality aspects. Therefore, only four genomes could be used for cgSNP typing. Interestingly, these isolates showed different MLST STs and did not cluster with the present strains isolated from poultry. The relevance of these findings is not clear. Various STs have been assigned to isolates from different governorates, with the exception of ST641, which is only present in isolates from the Al Gharbiyah governorates. A local trade or production structure may account for this finding. However, the numbers of isolates from each geographical region are too small to come to a final conclusion. The application of WGS to explore AMR and virulence in *P. aeruginosa* in Egypt is a necessary and useful tool for further clarification of the epidemiological links and One Health impact.

Further studies are required to estimate the potential transmission of *P. aeruginosa* between humans, animals and the environment in Egypt.

Funding

This work was supported internally by FLI. Tamara Kozytska was supported by a research grant from the VolkswagenStiftung Az: 9C071.

CRediT authorship contribution statement

Amira M. Rizk: Writing – review & editing, Investigation, Data curation. **Marwa M. Elsayed:** Methodology, Investigation, Data curation. **Ashraf A. Abd El Tawab:** Writing – review & editing, Supervision, Conceptualization. **Fatma I. Elhofy:** Writing – review & editing, Supervision, Conceptualization. **Enas A. Soliman:** Methodology, Data curation. **Tamara Kozytska:** Writing – original draft, Methodology, Formal analysis. **Hanka Brangsch:** Writing – review & editing, Software, Methodology, Formal analysis, Data curation. **Lisa D. Sprague:** Writing – review & editing, Validation, Formal analysis, Conceptualization. **Heinrich Neubauer:** Writing – review & editing, Validation, Supervision, Resources, Formal analysis, Data curation, Conceptualization. **Gamal Wareth:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization.

Declaration of Competing Interest

All authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetmic.2024.110063.

References

Abd El-Ghany, W.A., 2021. *Pseudomonas aeruginosa* infection of avian origin: zoonosis and one health implications. Vet. World 14 (8), 2155–2159. https://doi.org/10.14202/vetworld.2021.2155-2159. PubMed PMID: 34566334; PubMed Central PMCID: PMCPMC8448624.

Abdelatti, M.A.I., Abd El-Aziz, N.K., El-Naenaeey, E.Y.M., Ammar, A.M., Alharbi, N.K., Alharthi, A., Zakai, S.A., Abdelkhalek, A., 2023. Antibacterial and anti-efflux activities of cinnamon essential oil against pan and extensive drug-resistant *Pseudomonas aeruginosa* isolated from human and animal sources. Epub 2023/10/27 Antibiot. (Basel) 12 (10). https://doi.org/10.3390/antibiotics12101514.

Algammal, A.M., Mabrok, M., Sivaramasamy, E., Youssef, F.M., Atwa, M.H., El-Kholy, A. W., Hetta, H.F., Hozzein, W.N.J.Sr, 2020. Emerging MDR-*Pseudomonas aeruginosa* in fish commonly harbor oprL and toxA virulence genes and blaTEM, blaCTX-M, and tetA antibiotic-resistance genes. Sci. Rep. 10 (1), 15961 https://doi.org/10.1038/s41598-020-72264-4.

Algammal, A.M., Eidaroos, N.H., Alfifi, K.J., Alatawy, M., Al-Harbi, A.I., Alanazi, Y.F., Ghobashy, M.O.I., Khafagy, A.R., Esawy, A.M., El-Sadda, S.S., Hetta, H.F., El-Tarabili, R.M., 2023. oprL gene sequencing, resistance patterns, virulence genes, quorum sensing and antibiotic resistance genes of XDR *Pseudomonas aeruginosa* isolated from broiler chickens. Infect. Drug Resist 16, 853–867. https://doi.org/

10.2147/idr.S401473. PubMed PMID: 36818807; PubMed Central PMCID: PMCPMC9937075.
Ameen, F., Reda, S.A., El-Shatoury, S.A., Riad, E.M., Enany, M.E., Alarfaj, A.A.J.Sjobs, 2019. Prevalence of antibiotic resistant mastitis pathogens in dairy cows in Egypt and potential biological control agents produced from plant endophytic actinobacteria. Saudi J. Biol. Sci. 26 (7), 1492–1498. https://doi.org/10.1016/j.sjbs.2019.09.008. Epub 2019 Sep 14.
Argimón, S., Abudahab, K., Goater, R.J.E., Fedosejev, A., Bhai, J., Glasner, C., Feil, E.J., Holden, M.T.G., Yeats, C.A., Grundmann, H., Spratt, B.G., Aanensen, D.M., 2016. Microreact: visualizing and sharing data for genomic epidemiology and phylogeography. Micro Genom. 2 https://doi.org/10.1099/mgen.0.000093. PubMed PMID: 28348833; PubMed Central PMCID: PMCPMC5320705.
Badawy, B., Moustafa, S., Shata, R., Sayed-Ahmed, M.Z., Alqahtani, S.S., Ali, M.S., Alam, N., Ahmad, S., Kasem, N., Elbaz, E., El-Bahkiry, H.S., Radwan, R.M., El-Gohary, A., Elsayed, M.M., 2023. Prevalence of multidrug-resistant *Pseudomonas aeruginosa* isolated from dairy cattle, milk, environment, and workers' hands. Epub 2023/11/25 Microorganisms 11 (11). https://doi.org/10.3390/microorganisms11112775.
Baiomy, A.A., Serry, F.E., Kadry, A.A., Yahya, G., Doijad, S., Mostafa, A., Mraheil, M.A., El-Ganiny, A.M., 2023. Genome analysis of *Pseudomonas aeruginosa* strains from chronically infected patients with high levels of persister formation. Epub 2023/03/30 Pathogens 12 (3). https://doi.org/10.3390/pathogens12030426.
Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V.M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. Epub 2012/04/18 J. Comput. Biol. 19, 455–477. https://doi.org/10.1089/cmb.2012.0021.
Breidenstein, E.B., de la Fuente-Núñez, C., Hancock, R.E., 2011. *Pseudomonas aeruginosa*: All roads lead to resistance. Epub 2011/06/15 Trends Microbiol. 19, 419–426. https://doi.org/10.1016/j.tim.2011.04.005.
Caneschi, A., Bardhi, A., Barbarossa, A., Zaghini, A., 2023. The use of antibiotics and antimicrobial resistance in veterinary medicine, a complex phenomenon: a narrative review. Epub 2023/03/30 Antibiot. (Basel) 12 (3). https://doi.org/10.3390/antibiotics12030487.
Chen, L., Yang, J., Yu, J., Yao, Z., Sun, L., Shen, Y., Jin, Q., 2005. VFDB: a reference database for bacterial virulence factors. D325-328. Epub 2004/12/21 Nucleic Acids Res. 33. https://doi.org/10.1093/nar/gki008.
Curran, B., Jonas, D., Grundmann, H., Pitt, T., Dowson, C.G., 2004. Development of a multilocus sequence typing scheme for the opportunistic pathogen *Pseudomonas aeruginosa*. J. Clin. Microbiol. 42 (12), 5644–5649. https://doi.org/10.1128/jcm.42.12.5644-5649.2004. PubMed PMID: 15583294; PubMed Central PMCID: PMCPMC535286.
Dahshan, H., Abd-Elall, A.M., Megahed, A.M., Abd-El-Kader, M.A., Nabawy, E.E., 2015. Veterinary antibiotic resistance, residues, and ecological risks in environmental samples obtained from poultry farms, Egypt. Environ. Monit. Assess. 187 (2), 2. https://doi.org/10.1007/s10661-014-4218-3. PubMed PMID: 25600402.
Dapgh, A., Hakim, A., Abouelhag, H., Abdou, A., Elgabry, E.J.V.W., 2019. Detection of virulence and multidrug resistance operons in *Pseudomonas aeruginosa* isolated from Egyptian Baladi sheep. and goat 12 (10), 1524.
De Oliveira, D.M.P., Forde, B.M., Kidd, T.J., Harris, P.N.A., Schembri, M.A., Beatson, S.A., Paterson, D.L., Walker, M.J., 2020. Antimicrobial resistance in ESKAPE pathogens. Epub 2020/05/15 Clin. Microbiol. Rev. 33 (3). https://doi.org/10.1128/cmr.00181-19.
Edward, E.A., El Shehawy, M.R., Abouelfetouh, A., Aboulmagd, E., 2023. Prevalence of different virulence factors and their association with antimicrobial resistance among *Pseudomonas aeruginosa* clinical isolates from Egypt. Epub 2023/06/04 BMC Microbiol. 23, 161. https://doi.org/10.1186/s12866-023-02897-8.
Elshafiee, E.A., Nader, S.M., Dorgham, S.M., Hamza, D.A.J.Jo.V.R., 2019. Carbapenem-resistant Pseudomonas aeruginosa originating from farm animals and people in Egypt 63, 333.
Eraky, R.D., Abd El-Ghany, W.A., Soliman, K.M., 2020. Studies on *Pseudomonas aeruginosa* infection in hatcheries and chicken. J. Hell. Vet. Med. Soc. 71, 1953–1962.
Feldgarden, M., Brover, V., Haft, D.H., Prasad, A.B., Slotta, D.J., Tolstoy, I., Tyson, G.H., Zhao, S., Hsu, C.H., McDermott, P.F., Tadesse, D.A., Morales, C., Simmons, M., Tillman, G., Wasilenko, J., Folster, J.P., Klimke, W., 2019. Validating the AMRFinder tool and resistance gene database by using antimicrobial resistance genotype-phenotype correlations in a collection of isolates. Epub 2019/08/21. doi: Antimicrob. Agents Chemother. 63 (11) https://doi.org/10.1128/aac.00483-19.
Hamed, E.A., Abdelaty, M.F., Sorour, H.K., Roshdy, H., AbdelRahman, M.A.A., Magdy, O., Ibrahim, W.A., Sayed, A., Mohamed, H., Youssef, M.I., Hassan, W.M., Badr, H., 2021. Monitoring of antimicrobial susceptibility of bacteria isolated from poultry farms from 2014 to 2018. Epub 2021/09/21 Vet. Med. Int. 2021, 6739220. https://doi.org/10.1155/2021/6739220.
Hassan, W.H., Ibrahim, A.M.K., Shany, S.A.S., Salam, H.S.H.J.Joav, research, a, 2020. Virulence and resistance determinants in *Pseudomonas aeruginosa* isolated from pericarditis in diseased broiler chickens in Egypt. J. Adv. Vet. Anim. Res. 7 (3), 452–463. https://doi.org/10.5455/javar.2020.g441.
Holt, J.G., Krieg, N.R., Sneath, P.H., Staley, J.T., Williams, S.T., 1994. Bergey's Manual of determinate bacteriology.
Jadimurthy, R., Mayegowda, S.B., Nayak, S.C., Mohan, C.D., Rangappa, K.S., 2022. Escaping mechanisms of ESKAPE pathogens from antibiotics and their targeting by natural compounds. Epub 2022/06/11 Biotechnol. Rep. (Amst.) 34, e00728. https://doi.org/10.1016/j.btre.2022.e00728.
Jia, B., Raphenya, A.R., Alcock, B., Waglechner, N., Guo, P., Tsang, K.K., Lago, B.A., Dave, B.M., Pereira, S., Sharma, A.N., Doshi, S., Courtot, M., Lo, R., Williams, L.E., Frye, J.G., Elsayegh, T., Sardar, D., Westman, E.L., Pawlowski, A.C., Johnson, T.A., Brinkman, F.S., Wright, G.D., McArthur, A.G., 2017. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. D566-d573. Epub 2016/10/30 Nucleic Acids Res. 45. https://doi.org/10.1093/nar/gkw1004.
Kerr, K.G., Snelling, A.M., 2009. *Pseudomonas aeruginosa*: A formidable and ever-present adversary. Epub 2009/08/25 J. Hosp. Infect. 73, 338–344. https://doi.org/10.1016/j.jhin.2009.04.020.
Khater, D.F., Lela, R.A., El-Diasty, M., Moustafa, S.A., Wareth, G., 2021. Detection of harmful foodborne pathogens in food samples at the points of sale by MALDT-TOF MS in Egypt. Epub 2021/03/25 BMC Res. Notes 14, 112. https://doi.org/10.1186/s13104-021-05533-8.
Liao, C., Huang, X., Wang, Q., Yao, D., Lu, W., 2022. Virulence factors of *Pseudomonas Aeruginosa* and antivirulence strategies to combat its drug resistance. Front. Cell Infect. Microbiol. 12, 926758 https://doi.org/10.3389/fcimb.2022.926758. PubMed PMID: 35873152; PubMed Central PMCID: PMCPMC9299443.
Linde, J., Homeier-Bachmann, T., Dangel, A., Riehm, J.M., Sundell, D., Öhrman, C., Forsman, M., Tomaso, H., 2020. Genotyping of *Francisella tularensis* subsp. holarctica from Hares in Germany. Microorganisms 8 (12). https://doi.org/10.3390/microorganisms8121932. PubMed PMID: 33291395; PubMed Central PMCID: PMCPMC7761992.
Lister, P.D., Wolter, D.J., Hanson, N.D., 2009. Antibacterial-resistant *Pseudomonas aeruginosa*: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin. Microbiol. Rev. 22, 582–610. https://doi.org/10.1128/cmr.00040-09. PubMed PMID: 19822890; PubMed Central PMCID: PMCPMC2772362.
Low, A.J., Koziol, A.G., Manninger, P.A., Blais, B., Carrillo, C.D., 2023. ConFindr: rapid detection of intraspecies and cross-species contamination in bacterial whole-genome sequence data. PeerJ 7. https://doi.org/10.7717/peerj.6995. PMID: 31183253; PMCID: PMC6546082.
Mabrouk, S.S., Abdellatif, G.R., Zaid, A.S.A., Aboshanab, K.M., 2023. Propranolol restores susceptibility of XDR Gram-negative pathogens to meropenem and Meropenem combination has been evaluated with either tigecycline or amikacin. BMC Microbiol. 23, 195. https://doi.org/10.1186/s12866-023-02934-6. PubMed PMID: 37481513; PubMed Central PMCID: PMCPMC10362616.
Marouf, S., Li, X., Salem, H.M., Ahmed, Z.S., Nader, S.M., Shaalan, M., Awad, F.H., Zhou, H., Cheang, T., 2023. Molecular detection of multidrug-resistant *Pseudomonas aeruginosa* of different avian sources with pathogenicity testing and in vitro evaluation of antibacterial efficacy of silver nanoparticles against multidrug-resistant P. aeruginosa. Poult. Sci. 102, 102995 https://doi.org/10.1016/j.psj.2023.102995. PubMed PMID: 37566970; PubMed Central PMCID: PMCPMC10440575.
Mostafa, S.H., Saleh, S.E., Khaleel, E.F., Badi, R.M., Aboshanab, K.M., Hamed, S.M., 2023. Phenotypic and genotypic analysis of bacterial pathogens recovered from patients diagnosed with fever of unknown origin in Egypt. Antibiot. (Basel) 12 (8). https://doi.org/10.3390/antibiotics12081294. PubMed PMID: 37627714; PubMed Central PMCID: PMCPMC10451874.
Pang, Z., Raudonis, R., Glick, B.R., Lin, T.J., Cheng, Z., 2019. Antibiotic resistance in *Pseudomonas aeruginosa*: Mechanisms and alternative therapeutic strategies. Biotechnol. Adv. 37, 177–192. https://doi.org/10.1016/j.biotechadv.2018.11.013. PubMed PMID: 30500353.
Pelegrin, A.C., Palmieri, M., Mirande, C., Oliver, A., Moons, P., Goossens, H., van Belkum, A., 2021. *Pseudomonas aeruginosa*: A clinical and genomics update. FEMS Microbiol. Rev. 45 (6) https://doi.org/10.1093/femsre/fuab026. PubMed PMID: 33970247.
Sathe, N., Beech, P., Croft, L., Suphioglu, C., Kapat, A., Athan, E., 2023. *Pseudomonas aeruginosa*: Infections and novel approaches to treatment "Knowing the enemy" the threat of Pseudomonas aeruginosa and exploring novel approaches to treatment. Infect. Med. (Beijing) 2, 178–194. https://doi.org/10.1016/j.imj.2023.05.003. PubMed PMID: 38073886; PubMed Central PMCID: PMCPMC10699684.
Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033. PubMed PMID: 24451623; PubMed Central PMCID: PMCPMC3998144.
Stover, C.K., Pham, X.Q., Erwin, A.L., Mizoguchi, S.D., Warrener, P., Hickey, M.J., Brinkman, F.S., Hufnagle, W.O., Kowalik, D.J., Lagrou, M., Garber, R.L., Goltry, L., Tolentino, E., Westbrock-Wadman, S., Yuan, Y., Brody, L.L., Coulter, S.N., Folger, K.R., Kas, A., Larbig, K., Lim, R., Smith, K., Spencer, D., Wong, G.K., Wu, Z., Paulsen, I.T., Reizer, J., Saier, M.H., Hancock, R.E., Lory, S., Olson, M.V., 2000. Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. Nature 406, 959–964. https://doi.org/10.1038/35023079. PubMed PMID: 10984043.
Vidaillac, C., Chotirmall, S.H., 2021. Pseudomonas aeruginosa in bronchiectasis: infection, inflammation, and therapies. Epub 2021/03/20 Expert Rev. Respir. Med. 15, 649–662. https://doi.org/10.1080/17476348.2021.1906225.
Wareth, G., Melzer, F., Neubauer, H., 2017. In *Brucella*: selective pressure may turn some genes on instead of default off position. Med. Hypotheses 103, 29–31. https://doi.org/10.1016/j.mehy.2017.04.006. PubMed PMID: 28571803.
Wareth, G., Linde, J., Hammer, P., Pletz, M.W., Neubauer, H., Sprague, L.D., 2022. WGS-based phenotyping and molecular characterization of the resistome, virulome and plasmid replicons in *klebsiella pneumoniae* isolates from powdered milk produced in Germany. Epub 2022/03/27 Microorganisms 10 (3). https://doi.org/10.3390/microorganisms10030564.
Wood, D.E., Lu, J., Langmead, B., 2019. Improved metagenomic analysis with Kraken 2. Genome Biol. 20, 257. https://doi.org/10.1186/s13059-019-1891-0. PubMed PMID: 31779668; PubMed Central PMCID: PMCPMC6883579.

Yuan-Chuan, C. 2020. Introductory Chapter: Gene expression and phenotypic traits, In: Yuan-Chuan, C., Shiu-Jau, C. (Eds.) Gene Expression and Phenotypic Traits. IntechOpen, Rijeka, Ch. 1.

Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup, F.M., Larsen, M.V., 2012. Identification of acquired antimicrobial resistance genes. J. Antimicrob. Chemother. 67, 2640–2644. https://doi.org/10.1093/jac/dks261. PubMed PMID: 22782487; PubMed Central PMCID: PMCPMC3468078.

Zendri, F., Isgren, C.M., Devaney, J., Schmidt, V., Rankin, R., Timofte, D., 2023. Resistome-based surveillance identifies ESKAPE pathogens as the predominant gram-negative organisms circulating in veterinary hospitals. Front. Microbiol. 14, 1252216 https://doi.org/10.3389/fmicb.2023.1252216. PubMed PMID: 37744932; PubMed Central PMCID: PMCPMC10513425.