Prevalence, antibiotic profile, virulence determinants, ESBLs, and non-β-lactam encoding genes of MDR Proteus spp. isolated from infected dogs

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This study investigated the prevalence, antibiogram, virulence, extended-spectrum β-lactamases (ESBLs), and non-β-lactam encoding genes of Proteus species isolated from infected dogs in Ismailia province, Egypt. The study was conducted on 70 fecal swabs collected from dogs with diarrhea for bacteriological identification of Proteus spp. The positive isolates were evaluated for antibiotic susceptibility, molecular tests of virulence, ESBLs, and non-β-lactam encoding genes. Prevalence of Proteus spp. was 35.7% (25/70), including Proteus mirabilis (n = 23) and Proteus vulgaris (n = 2). The Proteus spp. prevalence revealed diversity, higher in males than females, in ages < 12 weeks. Investigation of antimicrobial resistance was found against penicillin and amoxicillin (100%), amoxicillin–clavulanic acid (32%), cephalosporins: cefotaxime and ceftazidime (36%), and monobactam: aztreonam (28%) as ESBLs, in addition to tetracycline (32%) and trimethoprim sulfamethoxazole (100%). The strains retrieved by PCR revealed ureC, zap, and rsbA virulence genes with variant prevalence as 92%, 60%, and 52%, respectively. In addition, the recovered strains contained ESBL genes with a dramatic variable prevalence of 100%, 92%, 36%, and 32%, to blaTEM, blaSHV, blaCTX-M, and blaOXA-1, respectively, and non-β-lactam encoding genes with a prevalence of 100%, 48%, 44%, 20%, and 12%, to sul1, tetA, intI1, qnrA, and aadA1. Moreover, 28% (7/25) of recovering strains were MDR (multidrug-resistant) up to four classes of antimicrobials, and 48% (12/25) of the examined strains were MDR up to three antimicrobial classes. In conclusion, to the best of our knowledge, our study could be the first report recording MDR Proteus spp. in dogs in Egypt.
1 Introduction

Proteus species are Gram-negative bacilli belonging to the Enterobacteriaceae family, which is widespread in both the human and animal gastrointestinal tracts, especially the members of the genus Proteus (Hegazy, 2016). It is the ultimate example of an opportunistic nosocomial pathogen (Jacobsen and Shirtliff, 2011). P. mirabilis, P. vulgaris, P. penneri, P. hauseri, P. terrae, and P. cibarii, with the unidentified genomic species, are included currently within the Proteus genus (Manos and Belas, 2006; Drzewiecka, 2016). P. mirabilis pathogens were isolated from humans, dogs, monkeys, pigs, sheep, goats, raccoons, cats, rodents, and other mammals. It is a considered a part of the normal microbiota of the mammalian intestinal tract (Guentzel, 1996). In addition, P. mirabilis and P. vulgaris are widely disseminated in the environment, occurring in contaminated water, sewage, and soil. It is critical to decompose the animals’ organic substances (Rózalski et al., 1997).

The antimicrobial-resistant strains of Proteus from companion animals were recorded. Their infectious nature results from their resistance to antipathogenic chemicals, enabling them to still be intact in their environment (Hola et al., 2012). The developed antimicrobial resistance leads to failure in treatment associated with an adverse impact on the animal’s welfare. Bacterial pathogens with resistance properties against antimicrobials can have substantial public health consequences for human beings if isolates are transmitted from pets to their owners (Guardabassi et al., 2004; Lloyd, 2007; Algammal et al., 2020). The resistance to extended-spectrum β-lactam is mostly related to the production of extended-spectrum β-lactamases; ESBLs would lyse many β-lactam antimicrobial agents like penicillins, different generations of cephalosporins, and carbapenems (Magiorakos et al., 2012; Rubin and Pitout, 2014; Algammal et al., 2020; Algammal et al., 2021b). The chemical backbone of β-lactam antibiotics is the β-lactam ring, which can be broken by these enzymes (Fadare and Okoh, 2021). The resistance of ESBL producers to many antibiotic classes is a major concern in clinical settings as it renders the treatment of individuals infected with these bacteria difficult and frequently impossible (Rawat and Nair, 2010). Resistance to sulfonamides usually develops primarily due to dihydropteroate synthase (DHPS) enzyme or their mutations carrying folP gene involved in the biosynthesis of the nucleotide or via the development of alternative genes of DHPS, such as sul1, sul2, and sul3, showing low affinity to sulfonamides (Perreten and Boerlin, 2003; Yun et al., 2012). Sulfonamides are chemotherapeutic drugs that act as competitive inhibitors of the folP gene-encoded DHPS. Widespread sulfonamide resistance is mostly caused by plasmid- and integron-borne sul1-3 genes that code encoding mutant DHPS enzymes that do not bind to sulfonamides (Sánchez-Osuna et al., 2019). Tetracycline-resistant genes are most commonly found on conjugated plasmids and transposons. On the other hand, other isolates have the necessary genes encoded on their chromosomes (Guillaume et al., 2000; Oppegaard et al., 2001). Efflux pumps, ribosome protection, and enzymatic deactivation are the critical mechanisms of tetracycline resistance acquired by acquiring tet genes. The aad A family of genes encodes aminoglycoside-3″-adenylyltransferase (AAD), which promote aminoglycosides (White and Rawlinson, 2001). The quinolone-resistant Enterobacteriaceae are growing worldwide. The qnr genes enhance resistance to nalidixic acid and reduce ciprofloxacin susceptibility (Crump et al., 2003). Class 1 integrons are frequently linked to multidrug resistance due to their ability to acquire or eliminate several antimicrobial resistance gene cassettes (Chen et al., 2017). Awareness of bacterial resistance against the antimicrobials, among the isolates of canine Proteus spp., is essential from a veterinary standpoint and an international public health perspective (Harada et al., 2014).

Currently, the pathogenic mechanism of Proteus-associated diarrhea remains unknown. It is necessary and useful to investigate the intestinal pathogenicity of Proteus, which can cause diarrhea. Few published studies have elucidated the pathogenic mechanism of Proteus diarrhea until now (Gong et al., 2019). Proteus spp. invasiveness is related to several virulent factors, and virulence genes encoded in plasmids regulate these virulent factors (Manos and Belas, 2006). Swarming on solid surfaces is a prominent characteristic property of Proteus spp. Although several genes were linked with swarming, the rsbA gene is a necessary swarming phenomenon for swarming control (Rather, 2005). Urease is an essential biomarker in Proteus infection for developing bladder and kidney stones. Many urease genes, like ureC, are essential to the urease enzyme development process (Li and Mobley, 2002). The zap gene product is encoded by many genes essential for protease production, especially zapA for regulating the expression of IgA protease during differentiation between the swimmer and swarmer cells (Walker et al., 1999). Urease and protease are considered diagnostic and differential aspects that describe this genus member from other Enterobacteriaceae members (Ali and Yousif, 2015).

Since the risky infection of Proteus spp. in dogs in Egypt has not been comprehensively clarified yet, this study aimed to clarify the prevalence, antibiogram, virulence genes, and ESBLs and...
non-β-lactam encoding genes of different Proteus spp. isolates to explore the potential hazard of Proteus spp. in dogs.

**2 Materials and methods**

**2.1 Ethical approval**

The study was conducted in accordance with the applicable rules and regulations of the Animal Ethics Review Committee of Suez Canal University (AERC-SCU) in Egypt. The experimental methods and laboratory work followed bacterial isolation, biosafety, and quality standards. In this study, no animals were experimentally used; however, dog handling and samples were applied with the owners’ consent.

**2.2 Study period and location**

This study has been performed in a private small animal clinic in the Ismailia governorate, Egypt, during the period from April to September 2020.

**2.3 Animals and clinical examination**

Moreover, 70 diseased puppies of different breeds and sex aged between 3–6 months were examined in our study. All the admitted dogs were suffering from diarrhea. Clinical examinations of such cases were performed as described by Côté et al. (2015). Fecal swabs placed in peptone water (Oxoid, United Kingdom) were randomly and aseptically
obtained from the diseased animals and transmitted directly to the laboratory for bacteriological examination.

2.4 Isolation of *Proteus* spp. for identification

The swabs were supplemented in peptone water (Oxoid, United Kingdom) for 24 h at 37°C, then enriched broth was passed on XLD (xylose lysine deoxycholate) agar, 5% sheep blood agar, MacConkey agar, and TSI agar (triple sugar iron) (Oxoid, United Kingdom) by using a sterile bacterial loop, and then incubated at 37°C for 24–48 h. Bacterial identification was performed based on the culture characteristics, swarming activity, hemolytic activity, morphological characteristics with Gram’s staining, and suspicious colonies of bacteria were biochemically identified by catalase, H₂S generation, urease, methyl red, citrate utilization, oxidase, and lactose fermentation. As previously stated, the indole test was applied to differentiate between *P. mirabilis* and *P. vulgaris* (Quinn et al., 2002).

### Table 2 Odds ratio of *Proteus* isolates among age, sex, and breed.

|     | OR     | 95% CI          | Chi-squared | p-value |
|-----|--------|-----------------|-------------|---------|
| Age | 1.193489 | 0.7001873–2.108472 | 4.4707 | 0.2149 |
| Sex | 1.5000000 | 0.4368678–5.420620 | 0.10686 | 0.7437 |
| Breed | 0.9386627 | 0.7519946–1.165913 | 9.0488 | 0.3382 |

2.4.1 Susceptibility of the antimicrobial agents

Antimicrobial susceptibility for *Proteus* spp. isolates was performed using the Mueller–Hinton agar (Oxoid, United Kingdom) involved in the disc diffusion method, in which 11 (n = 11) antimicrobial agents were used: amoxicillin (AMX; 10 μg), penicillin (P; 10 U), amoxicillin–clavulanic acid (AMC; 30 μg), cefazidime (CAZ; 30 μg), cefotaxime (CTX; 30 μg), aztreonam (ATM; 30 μg), norfloxacin (NOR; 10 μg), nalidixic acid (NA; 30 μg), gentamicin (gen; 10 μg), tetracycline (TE; 10 μg), and sulfamethoxazole–trimethoprim (SXT; 25 μg) (Oxoid, United Kingdom). *E. coli*-ATCC 35218 was used as a quality control strain. The isolates were tested for ESBLs using cephalosporin indicators CAZ (30 μg) and CTX (30 μg) (Oxoid, United Kingdom). The diameter of inhibition zones was calculated as < 22 mm and < 27 mm, respectively (CLSI, 2017). The phenotype resistance patterns were classified as pan drug-resistant (PDR) and are resistant to all used antimicrobial agents; extensive drug-resistant (XDR) species is resistant to one agent in all but two antimicrobial classes, multidrug-resistant (MDR) species is resistant to one agent in three antimicrobial classes or drug-resistant (DR) species is resistant to less than one agent in three antimicrobial classes as stated by Magiorakos et al. (2012). The multiple antibiotic resistance index (MARI) was calculated following this mathematical equation: MARI = c/d. The “c” indicates the sum of the antimicrobial agents against which the bacteria exhibited resistance, while “d” indicates the total of those agents used.
2.4.2 DNA extraction

Bacterial genomic DNA was extracted from the retrieved Proteus spp. isolates using the QIAamp DNA Mini Kit (QIAGEN Sciences Inc., Germantown, MD, United States/Cat. No. ID 51326) according to the manufacturer’s instruction manual.

2.4.3 Virulence-determinant, extended-spectrum β-lactamases, and non-β-lactam encoding gene detection

Moreover, 25 positive isolates of DNA extracts were used for virulence-determinant genes by using the PCR targeting (ureC, zapA, and rshA) genes, as previously conducted (Pathirana et al., 2018); ESBL genes (bla<sub>TEM</sub>, bla<sub>CTX-M</sub>, bla<sub>SHV</sub>, and bla<sub>XXA</sub>), as previously mentioned (Colom et al., 2003; Sharma et al., 2009); and non-β-lactam encoding genes for sulfonamides (sul1), tetracyclines (tetA), aminoglycosides (aadA1), quinolones (qnrA), and integrons (intI1), as previously illustrated (White et al., 2000; Randall et al., 2004; Robicsek et al., 2006; Ibekwe et al., 2011). All genes’ detection of PCR experiments in this study were carried out with a 50 μl total reaction volume as 5 μl of PCR buffer (10×), 1 μl 200 μM (from each dNTP of 10 mM dNTP mix), 4 μl of the bacterial template, 0.4 μl Taq DNA polymerase (5 U/L), 30 pmol per primer, and H₂O was added up to 0.05 ml. The PCR cycling conditions and oligonucleotide primers (Thermo Fisher Scientific, United States) are presented in Table 1. Positive control strains, provided by the Animal Health Research Institute (AHRI), Dokki, Egypt, and the negative template control (NTC) were used in all assays. The amplified PCR products were visualized against a 100-pb DNA marker by agar gel electrophoresis on 1.5% agarose containing ethidium bromide 0.5 g/ml.

2.4.4 Statistical data analyses

Data were analyzed by the chi-squared test according to Feldman et al. (2008) by R software (version 4.0.2, www.r-project.org), and the differences between groups were considered significant at p < 0.05. Visualization of Proteus isolates according to the virulence and resistance genes with a heatmap supported by hierarchical clustering (dendrogram) was performed according to Kolde (2019), and cor and ggcorrplot packages were used to identify the correlation among the various antimicrobial agents. Fisher’s exact test and odds ratio [confidence interval (CI) = 0.95%] were determined among sex, age, and breed of examined dogs.

3 Results

3.1 Clinical manifestation presentation in dogs

All examined dogs showed an increased body temperature higher than or equal to 39.5°C, lethargy, anorexia, depression, dehydration, and foul-smelling watery bloody diarrhea. The record increase in body temperature progressed to subnormal later in the late stage of disease; also, the intensity of dehydration increased in parallel to diarrhea.

3.2 Phenotype and prevalence of the Proteus spp. in examined samples

Moreover, 25 Proteus spp. isolates were isolated from 70 fecal swabs obtained from sick dogs exhibiting diarrhea, depending on the phenotypic characteristics of colonies, microscopic morphology, and biochemical markers. On XLD, the colonies were red with a black center but appeared pale (non-lactose fermenter) on MacConkey agar, black on TSI (H₂S producer), and hemolytic on the blood agar with clearly detected swarming activity. The biochemical tests of those isolates were positive for catalase, H₂S generation, urease, methyl red, and citrate utilization. In contrast, a negative response to oxidase, lactose fermentation, and indole was detected, but the Voges–Proskauer tests were positive for Proteus spp. The indole test was used to differentiate between P. vulgaris and P. mirabilis as it was positive for P. vulgaris and negative for P. mirabilis. The overall prevalence of Proteus spp. was 35.7% (25/70) as 92% (23/25) vs. 8% (2/25) for P. mirabilis and P. vulgaris, respectively. In addition, 25.7% of the examined diseased animals (n = 18) are infected with other bacterial pathogens, including, E. coli (13/70, 18.6%), Klebsiella pneumoniae (4/70, 5.7%), and Pseudomonas aeruginosa (1/70, 1.4%).
### Table 3: Antibiotic-resistant phenotypes of the recovered isolates of Proteus.

| Antibiotic class                        | Tested antibiotic                      | Interpretation |
|-----------------------------------------|-----------------------------------------|----------------|
|                                         | Sensitive                              | Intermediate  | Resistance |
|                                         | N | % | N | % | N | % |
| β-Lactam-β-lactamase inhibitor combinations | Penicillins | — | — | — | — | 25 | 100 |
|                                         | Amoxicillin | — | — | — | — | 25 | 100 |
|                                         | Amoxicillin-clavulanic acid | 4 | 16 | 13 | 52 | 8 | 32 |
| Cephalosporins                          | Cefotaxime | 12 | 48 | 4 | 16 | 9 | 36 |
|                                         | Ceftazidime | 11 | 44 | 5 | 20 | 9 | 36 |
| Monobactam                              | Aztreonam | 11 | 44 | 7 | 28 | 7 | 28 |
| Aminoglycosides                         | Gentamicin | 2 | 8 | 20 | 80 | 3 | 12 |
| Quinolones                              | Nalidixic acid | 7 | 28 | 15 | 60 | 3 | 12 |
| Fluoroquinolones                        | Norfloxacin | 10 | 40 | 13 | 52 | 2 | 8 |
| Tetacycline                             | Tetacycline | 5 | 20 | 8 | 32 | 12 | 48 |
| Sulfonamides                            | Trimethoprim-Sulamethoxazole | — | — | — | — | 25 | 100 |
| Chi-square—p-value                      | 40.903 | 59.553 | 71.031 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
3.3 Risk factors for *Proteus* spp. prevalence in dogs

Prevalence of *Proteus* spp. varied with sex, age, and breed of examined dogs. It was higher in males, 64% (16/25) than in females 36% (9/25). It showed 60% (15/25) in dogs less than 12 weeks old rather than those of 4-, 5-, and 6 months old; 16% (4/25), 4% (1/25), and 20% (5/25), respectively. Concerning breeds, the prevalence rates showed 32% (8/25) in German Shepherd, 20% (5/25) in Rottweiler, 12% (3/25) in both Golden Retriever and Cane Corso, 8% (2/25) Husky, 8% (2/25) Griffon, and 8% (2/25) Pitbull. Statistically, no significant difference was detected among the retrieved isolates according to age, gender, and breed, as exhibited in Table 2 and Figure 1.

3.4 Antimicrobial-resistant phenotypes of retrieved strains

Susceptibility of the antimicrobial agents of the recovered *Proteus* spp. isolates revealed resistance to penicillins: penicillin and amoxicillin (100%), amoxicillin and clavulanic acid (32%); cephalosporins: cefotaxime and ceftazidime (36%); and monobactam: aztreonam (28%) as extended-spectrum β-lactamases, tetracycline (32%), and sulfonamides: trimethoprim sulfamethoxazole (100%), while both gentamicin and nalidixic acid showed low resistance (12%), as shown in Table 3; Figure 2. A significant difference was detected in the resistance of recovered isolates to the antibiotics tested ($p < 0.05$).

3.5 Antimicrobial agents’ correlation

Positive correlations were detected between amoxicillin, penicillin, and trimethoprim–sulfamethoxazole, between cefotaxime and ceftazidime, and also between cefotaxime, ceftazidime, and amoxicillin and clavulanic acid that confirm the relation between ESBL agents and finally between nalidixic acid, norfl Roxacin, and gentamicin, as illustrated in Figure 3.

### TABLE 4 Virulence-determinant gene prevalence of recovered isolates of *Proteus*

| Gene type | N    | %   | Chi-square—$p$-value |
|-----------|------|-----|----------------------|
| Virulence-determinant genes | ureC  | 23  | 92 | 3.2941—0.1926NS |
|            | zapA | 15  | 60 | |
|            | rsbA | 12  | 52 | |
3.6 Distribution of the virulence-determinant factors among the examined isolates

PCR confirmed that those examined strains harbored three virulence genes, ureC, zapA, and rsbA with variable prevalence as 92% (23/25), 60% (15/25), and 52% (13/25), respectively (Table 4; Figure 4). No significant difference was detected in prevalence of the virulence-determinant genes among the different Proteus spp. ($p < 0.05$).

3.7 Detection of potential extended-spectrum β-lactamase genes

Phenotypically, ESBLs showed 32% (9/25) of the recovered isolates. However, the genes of antimicrobial resistance, $bla_{TEM}$, $bla_{SHV}$, $bla_{CTX-M}$, and $bla_{OXA-1}$, as detected in the isolates by PCR with specific predicted bands, were successfully amplified, proving that the examined strains contained ESBL genes with a dramatic variable prevalence as 100% (25/25), 92% (22/25),
Significant differences among the ESBL gene prevalence of the recovered strains were detected \((p < 0.05)\). \(3.8\) Detection of integron class 1 and non-extended-spectrum \(\beta\)-lactamase genes

The integron \((\text{intI1})\) was detected at a prevalence of 44\% \((11/25)\), and the sulfonamide antimicrobial resistance gene \((\text{sul}1)\) detected in \(\text{Proteus}\) spp. isolates showed a prevalence of 100\% \((25/25)\), while the tetracycline resistance gene \((\text{tet}A)\) was detected at a prevalence of 48\% \((12/25)\), the quinolone resistance gene \((\text{qnr}A)\) was detected at a prevalence of 20\% \((5/25)\), and the aminoglycoside resistance gene \((\text{aad}A1)\) was detected in a prevalence of 12\% \((3/25)\) \((\text{Table 5; Figure 4})\). A significant difference was recorded in the sulfonamide or tetracycline gene prevalence in the recovered strains.

\textbf{3.9} The relation between the phenotypic- and genotypic-resistant patterns among \textit{Proteus}\textit{ }spp. isolates

The present findings found that 28\% \((7/25)\) of recovered isolates were multidrug-resistant to five antimicrobial families, penicillins: amoxicillin and ampicillin, cephalosporins: cefotaxime and ceftazidime, \(\beta\)-Lactam-\(\beta\)-lactamase inhibitor combination: amoxicillin–clavulanic acid, sulfonamides: trimethoprim–sulfamethoxazole, and tetracyclines: tetracycline, in response to the following resistance genes \(\text{bla}_{\text{TEM}}, \text{bla}_{\text{SHV}}, \text{bla}_{\text{OXA-1}}, \text{bla}_{\text{CTX-M}}, \text{and sul}1\). In addition, 48\% \((12/25)\) of those isolates were resistant to three antimicrobial families: penicillins: amoxicillin and ampicillin, sulfonamides: trimethoprim–sulfamethoxazole, and also tetracyclines: doxycycline, in response to \(\text{bla}_{\text{TEM}}, \text{bla}_{\text{SHV}}, \text{tet}A, \text{and sul}1\) genes. Furthermore, 12\% \((3/25)\) of the tested strains revealed MDR to four antimicrobial families: aminoglycoside: gentamicin, penicillins: amoxicillin and
ampicillin, quinolones; nalidixic acid, and sulfonamides: trimethoprim–sulfamethoxazole, with the resistant genes such as bla_{TEM}, bla_{SHV}, and sul1 (Table 6; Figure 4). Multiple antibiotic resistance index (MARI) values in this study (≥0.2) exhibited resistance patterns of recovered isolates obtained from a high-risk contamination level (Table 6).

**4 Discussion**

Studies in human medicine investigated the microbial activities of Proteus (Chen et al., 2012; Decôme et al., 2020) but still need to be unveiled in veterinary medicine (Wong et al., 2015). The present study is a novel investigation of prevalence, PCR-based virulence, antimicrobial drug resistance, and ESBL genes contributing to pathogenicity of the Proteus spp. isolated from canine fecal samples in Egypt. Few studies are concerning the emergency of the Proteus spp. in canines. In this study, the overall prevalence of Proteus spp. in feces of clinically diseased dogs was 37.5%. Two species were identified, with the most prevalent species being *P. mirabilis*, followed by *P. vulgaris*. This is less than the 13.6% infection rate of Proteus spp. in dogs with urinary tract diseases in Thailand (Amphaibhan et al., 2021). In another study, *P. mirabilis* was detected in 22.7% of dogs’ urinary samples in the United Kingdom (Fonseca et al., 2021). In addition, domestic dogs in Nigeria had a 28% prevalence of Proteus spp. in their feces (Obioma et al., 2020). The phenotypic attributes of recovered Proteus spp. strains were unambiguous and revealed a high degree of agreement between them: red-colored and centrally black colonies on the XLD agar, pale colored and non-lactose fermenter on the MacConkey agar, black colonies on TSI agar, and characteristic swarming activity of the colonies (characteristics for Proteus spp.). Catalase, H$_2$S, urease, methyl red, and citrate utilization are all positive for the recovered isolates. However, oxidase, lactose fermentation, Voges–Proskauer, and indole tests are all negative, except for *P. vulgaris*, which is positive for indole. Our results agreed with findings reported by Reich et al. (2013) and Lei et al. (2016). The conventional biochemical tests are necessary for the differentiation of Proteus and *Morganella*, and some tests are used for differentiation between them as swarming is characteristic for Proteus spp. and also H$_2$S (O’Hara et al., 2000).

According to our findings, the recovered isolates showed resistance to penicillins, cephalosporins, monobactams (extended-spectrum β-lactamases), sulfonamides, and tetracycline, while both gentamicin and nalidixic acid showed low resistance. MARI values were ≥ 0.2, so it exhibited multiple resistance patterns, denoting that the recovered isolates were taken from high-risk contamination and that *P. mirabilis* from dogs’ isolates has high MARI (Zhang et al., 2018). Small animal bacterial infections were frequently treated with broad-spectrum antimicrobials such as penicillins and tetracyclines. The antimicrobials’ misuse in small animals and the ability of *P. mirabilis* to acquire antimicrobial-resistant genes from other pathogens are the main causes of MDR strains (Algammal et al., 2021b).

Unfortunately, antimicrobial resistance was more common in *P. mirabilis* from dogs compared to those in human isolates (Siebor and Neuwirth, 2013). Prevalence of ampicillin resistance increased than predicted (71%) (Harada et al., 2014), which was most likely caused by TEM penicillinases (Hordijk et al., 2013). Resistance to trimethoprim/sulfamethoxazole, quinolones, and aminoglycosides to *P. mirabilis* restricts the therapeutic options of such antimicrobials used in the treatment of dogs infected with *P. mirabilis* (Wong et al., 2015).

The PCR proved that the recovered Proteus spp. strains from dogs are highly virulent as they harbored *ureC*, *zap*, and *rsb* virulence genes exhibiting prevalence as 92, 60, and 52%, respectively. The *ureC* gene causes urine pH to rise, resulting in the formation of stones (Armbruster et al., 2018). The Proteus spp. demonstrated in our study had 92% *ureC* amplification, indicating a higher frequency of *ureC* than other genes, thus playing a pivotal role in the virulence of Proteus depending on this gene. The *ureC* gene was identified in approximately 96.6% of human-infected urinary tract isolates (Ali and Yousif, 2015). In previous studies from ducks, the *ureC* gene was found in 100% of Proteus isolates (Algammal et al., 2021b). In addition, Proteus spp. lacked *rsb* gene which encodes the characteristic swarming activity of Proteus and expresses a membrane sensor for promoting the extracellular polysaccharides (Walker et al., 1999; Algammal et al., 2021a). The current report showed that 15 isolates (60%) had the *zap* gene, which codes for protease enzyme production, and *Zap*-protease could degrade IgG, IgA1, and IgA2, controlled by the *zap* gene (Pathirana et al., 2018; Algammal et al., 2021b).

When ESBLs are present, infectious bacteria develop resistance to lactamase, demonstrating the negative effect of the variant therapeutics (Schultz et al., 2017). ESBL-positive and -negative isolates showed different AMR rates. The mechanism of increased non-β-lactam resistance in ESBL-producing strains is unknown. ESBLs hydrolyze broad-spectrum lactam antibiotics, such as penicillins and cephalosporins in addition to piperacillin. Enterobacteriaces frequently produce ESRLs. According to our results, all ESBL-positive isolates had *bla_{TEM}* gene. *P. mirabilis*, identified in the hospitalized patients' samples, frequently contained ESBLs of the TEM type (Ahn et al., 2017; Rajivgandhi et al., 2018). The *bla_{CTX-M}* gene also exhibits resistance to cephalosporins, cefazidime, and cefotaxime. The *bla_{SHV}* gene is commonly detected in members of the Enterobacteriaces family (Alonso et al., 2017), which confirms our findings. Furthermore, according to our findings, the OXA-
1-positive isolates showed a significantly higher prevalence than that stated in a French study (Bonnet et al., 2002). The blaOXA-1 gene promotes piperacillin and cephalosporin resistance. The blaOXA-1 and blaCTX-M genes work together protecting P. mirabilis from lactam-lactamase inhibitor combinations (Schultz et al., 2017). Concurrently, Proteus is also resistant to sulfonamides and tetracyclines due to sul1 and tetA genes (Cadena et al., 2018; Algammal et al., 2021a). Class 1 integrons were connected with ESBL-producing isolates more frequently than non-ESBL-producing isolates (Chen et al., 2017). Some researchers suggest that the ESBL genes and integron are frequently connected with the qnr gene (Hopkins et al., 2007; Sharma et al., 2009). When bacteria are subjected to selective pressure by sulfonamides, the presence of sul1 genes with class 1 integrons provides a useful tool for the maintenance and subsequent development of resistance to other antimicrobial agents (Antunes et al., 2005).

Animal companions could be as reservoirs of Proteus, especially P. mirabilis, for human infection according to previous research that found human and animals infections with closely-related bacterium strains (Marques et al., 2019). P. mirabilis showed high resistance levels against the antimicrobial agents, making it a high elimination frequency through diarrhea in dogs.

5 Limitations and future recommendations

Future work is recommended to perform appropriate identification by molecular techniques like MALDI-TOF/16S rRNA gene sequencing to realize the clonal relatedness of the obtained strains.

6 Conclusion

Eventually, this report study could be the first study of MDR in Proteus spp. isolated from Egyptian dogs. P. mirabilis was a highly recorded prevalence in dogs, which was associated with diarrhea. The ureC, zapA, and rsbA virulence genes are commonly detected in the Proteus isolates obtained from infected dogs. The isolated Proteus strains showed multidrug resistance (MDR) property against penicillins, β-lactamases, sulfonamides, cephalosporins, quinolones, aminoglycosides, and tetracyclines. They often carried the blaOXA-1, blaTEM, blaCTX-M, blaSHV, intI1, tetA, aadA1, qnrA, and sul1 resistance genes. The potential hazards and health risks of the Proteus infection in dogs need more investigation. Therefore, we encourage continuous epidemiological data collection with the antimicrobial testing of susceptibility in both humans and animals and testing variant antibiotics used in human or veterinary medicinal fields, either.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials; further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by the Animal Ethics Review Committee of Suez Canal University (AERC-SCU) in Egypt.

Author contributions

RE-T and AM: conceptualization and study design. RE-T, AM, and EA: conducted the experiments. TE, AM, and RE-T: drafted the manuscript. RE-T, AHA, and AEA: carried out the statistical analysis and investigation. NA, DN, SKA, AM, AHA, and EME: data validation, data accuracy, and supervision. AM and TE: performed the clinical examination of diseased cases and sampling. RE-T, AHA, AM, SKA, and TE: wrote the original draft. EA, DN, SKA, and NA: critically revised the manuscript. All authors have revised and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer RE declared a shared affiliation with the authors DN and TE to the handling editor at the time of review.
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