Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2019.101

RESEARCH ARTICLE

Prevalence, Genetic Diversity and Antimicrobial Resistance of *Proteus mirabilis* Isolated from Dogs Hospitalized in Beijing

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ARTICLE HISTORY (19-271)

Received: June 23, 2019
Revised: August 20, 2019
Accepted: August 22, 2019
Published online: September 17, 2019

Key words:
Antimicrobial resistance
Dogs
ESBL
PFGE
Proteus mirabilis
RAPD

A B S T R A C T

Proteus mirabilis is an opportunistic pathogen that causes diarrhea and urinary tract infections in humans and companion animals. Emergence of antimicrobial resistance in *P. mirabilis* increases risk of failure of antimicrobial therapy in companion animals. The current study determined prevalence, genetic diversity and phenotypic antimicrobial resistance of *P. mirabilis* isolated from feces of 35 dogs with diarrhea hospitalized in Beijing, China. Random amplified polymorphic DNA (RAPD), pulse-field gel electrophoresis (PFGE), PCR and antimicrobial disk diffusion were used to characterize these isolates. Prevalence of *P. mirabilis* in feces from hospitalized dogs was 28%. RAPD and PFGE demonstrated a great diversity of isolates. Thirteen (37%) of 35 isolates produced extended-spectrum beta-lactamases (ESBLs), with bla_{TEM} being detected in all ESBLs-producing isolates. All isolates were susceptible to imipenem, cefoxitin and cefotaxime/clavulanic. *rmtB* (with 51% prevalence rate) was the only aminoglycoside 16S rRNA methylase resistance gene identified. Among plasmid-mediated quinolones resistance genes, *qnrB* (14%) and *aac(6')-Ib* (26%) were detected. In conclusion, *P. mirabilis* isolates from feces of dogs with diarrhea in China had great genetic diversity and a high prevalence produced ESBLs. Consequently, antimicrobial stewardship programs should also target companion animals to reduce emergence of antimicrobial resistance.

To Cite This Article: Han P, Luo L, Shahid M, Li M, Wang S, Nobrega DB, Sabir N, Barkema HW, Han B and Gao J, 2020. Prevalence, genetic diversity and antimicrobial resistance of *proteus mirabilis* isolated from dogs hospitalized in Beijing. Pak Vet J, 40(1): 61-66. http://dx.doi.org/10.29261/pakvetj/2019.101

INTRODUCTION

The genus *Proteus* belongs to the *Enterobacteriaceae* family. Bacteria in this genus are motile, Gram-negative and can thrive in soil, water and intestinal tracts of mammals (Drzewiecka, 2016). Several species of this genus are known to colonize and infect human and animal hosts, with *Proteus mirabilis* being the most prevalent one (Wang *et al*., 2014; Yazdi *et al*., 2018).

Proteus mirabilis has gained increasing importance as an emerging opportunistic pathogen causing nosocomial infections in humans and animals (Jacobsen and Shirtliff, 2011; Adams-Sapper *et al*., 2012). In companion animals, they have been implicated as causative agents of urinary tract infections (UTIs) and chronic otitis (Zamankhan Malayeri *et al*., 2010; Marques *et al*., 2019). There is supporting evidence that human patients with UTIs caused by *P. mirabilis* will have the same strain in their stool (Schaffer and Pearson, 2015). Likewise, presence of *Proteus* spp. in fecal content of dogs is associated with an increased risk of UTIs caused by the same bacteria (Harada *et al*., 2014). However, whether there is any genetic similarity between isolates that colonize dogs is apparently unknown. Therefore, identification of major clonal complexes colonizing dogs would provide valuable insights into the epidemiology of UTIs and other diseases caused by this important opportunistic pathogen.

In general, infections caused by *P. mirabilis* are treated with extended-spectrum cephalosporins (ESC), although aminoglycosides or fluoroquinolones are also used. Recently, the emergence of extended-spectrum beta-lactamases (ESBLs) in *P. mirabilis* has become an

increasing public health concern (Schultz *et al*., 2017). Whereas it is still uncertain whether and how these bacteria promote dissemination of antimicrobial resistance (AMR), ESBL-producing isolates may be clinically resistant to beta-lactams such as penicillin, aztreonam and cephalosporins (Lin *et al*., 2019). Currently, prevalence of AMR and associated genes in *P. mirabilis* isolated from hospitalized dogs in China with diarrhea remains unknown. In this study, we aimed to investigate prevalence, genetic diversity and AMR profile of *P. mirabilis* isolated from fecal samples of hospitalized dogs with diarrhea. In addition, presence of ESBL-encoding genes in *P. mirabilis* was also determined.

MATERIALS AND METHODS

Ethics statement: This study was conducted following ethical guidelines of China Agricultural University (CAU), Beijing. Prior to start of the study, ethical approval was granted by the Departmental committee of College of Veterinary Medicine, CAU. Sampling was carried out according to standardized protocols (Jay-Russell *et al*., 2014) and with prior consent of each owner.

Collection of samples, isolation and identification of Proteus mirabilis isolates: A total of 125 fecal samples was collected aseptically from 125 randomly selected hospitalized dogs with diarrhea at the Small Animal Teaching Hospital, College of Veterinary Medicine, China Agricultural University, from October 2014 to May 2015. A cotton swab was used to inoculate each sample on MacConkey agar (DifcoTM, Becton Dickinson, Sparks, MD USA). Bacterial identification was done based on colony characteristics, triple sugar iron test and gram staining, and isolates were confirmed to be *P. mirabilis* by API20E biochemical test (BioMerieux, Lyon, France).

DNA extraction and species confirmation: Bacterial genomic DNA was extracted according to manufacturer's instructions (TIANGEN, Beijing, China). Thereafter,16S rRNA was amplified by using forward Q-248-F 5-CGCAAGGATCAGACAGTCTCTCTCA-3 and reverse Q-248-R 5-CCTGTTAAGTTATC TCCTGAGTGAAT-3 primers (Beijing Sunbiotech Co. Ltd., Beijing, China) with an expected product size of 248 bp (Luo *et al*., 2008).

RAPD genotyping: For RAPD genotyping, *OPX13* 5'-ACGGGAGCAA-3 primers (Beijing Sunbiotech) were used as reported (Michelim *et al*., 2008). Amplifications were repeated three times (independent DNA extractions and PCR) to evaluate reproducibility for each isolate.

PFGE fingerprinting analysis: Genotyping of *P. mirabilis* isolates was conducted by macro restriction, using *XbaI* digestion of bacterial DNA, followed by separation of resulting fragments by PFGE. Resulting maps were prepared using Quantity OneTM software and cluster analysis performed using the UPGMA method based on Dice coefficients, with optimization and tolerance set at 0.5%. Analyses were done in BioNumerics V 5.1 software. Clusters were defined at the 80% similarity level. Degree of relatedness was considered as defined (Tenover *et al*., 1995).

Antimicrobial susceptibility testing: Antimicrobial susceptibility of all *P. mirabilis* isolates was done using the disc diffusion method, in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (2015). We tested the following antimicrobials: ampicillin (10 µg), ampicillin/sulbactam (10 µg), aztreonam (30 µg), piperacillin (30 µg), mezlocillin (75 µg), imipenem (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), cefotaxime/sulbactam (30/30 µg), amikacin (30 µg), gentamicin (10 µg), tobramycin (10 µg), chloramphenicol (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), ciprofloxacin (0.5 µg) and norfloxacin (10 µg). *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were used as quality-control strains.

ESBLs phenotyping: All isolates were screened for presence of ESBLs using the disk diffusion method (CLSI, 2015). Briefly, an ESBLs producer was detected with discs containing ceftazidime (CAZ, 30 µg) or cefotaxime (CTX, 30 µg) alone or in combination with clavulanic acid (CLA, 10 µg). Production of ESBLs was confirmed by an increase of ≥5 mm in zones of inhibition of combined disks when compared to agents tested alone.

Detection of ESBLs genes: Primers targeting ESBL genes (bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M\text{-}1}$, bla_{OXA1}) were synthesized (Beijing Sunbiotech Co., Ltd; Table 1) and reactions done in a final volume of 25 µl of PCR mixture for each sample, following references in Table 1.

Identification of antimicrobial resistance genes: All isolates were screened for presence of AMR genes (Table 2). PCR conditions were used as described (Kim *et al*., 2005). PCR-positive products were sequenced and compared to existing databases for further confirmation.

Statistical analyses: Confidence intervals (CI) were estimated using q binomial distribution. Fisher's Exact test was used to compare antimicrobial resistance rates between antimicrobials, and also between ESBL-producers and non-producers. Statistical significance was considered at the 5% level.

RESULTS

Samples collected and prevalence of Proteus mirabilis: A total of 35 *Proteus mirabilis* were isolated from 125 fecal samples based on colony characteristics, microscopic appearance, biochemical tests and 16S rRNA, with an estimated prevalence of 28% (95% exact confidence interval: 20-37%). Round transparent or translucent colonies with smooth surface and migrating growth at colony edges were visualized on SS agar plate (Fig. 1-A). Colonies were round, smooth and black on HE agar plates (Fig. 1-B). Hydrogen sulfide and gas-producing characteristics of *Proteus mirabilis* were readily detected in triple sugar iron testing (Fig. 1-C). In addition, when examined with an optical microscope, they were gram negative, polymorphic or rod-shaped bacilli, without spore or capsule formation (Fig. 1-D).

Table 1: Primers used to detect ESBLs resistance genes

Gene	Name	Primer (5'-3')	Product size (bp)	Reference
bla$_{TEM}$	TEM-A	TGCGGTATTATCCCGTGTTG	297	Hu, 2007
	TEM-B	TCGTCGTTTGGTATGGCTTC		
bla$_{SHV}$	SHV-A	TCTCCCTGTTAGCCACCCTG	593	
	SHV-B	CCACTGCAGCAGCTGCCGTT		
bla$_{CTX-M-1}$	CTX-1-A	ACAGCGATAACGTGGCGATG	197	
	CTX-1-B	CACCCAATGCTTTACCCAG		
bla$_{OXA1}$	OXA-1-A	TTTTCTGTTGTTTGGGTTTT	520	
	OXA-1-B	TTTCTTGGCTTTTATGCTTG		

Table 2: Primer used to identify antimicrobial resistance genes

Gene	Sequence (5'-3')	Product size (bp)	Reference
rmtB	F : TCAACGATGCCCTCACCTC	459	Fritsche *et al*., 2008
	R : GCAGGGCAAAGGTAAAATCC		
rmtC	F : GCCAAAGTACTCACAAGTGG	752	Fritsche *et al*., 2008
	R : CTCAGATCTGACCCAACAAG		
armA	F : TATGGGGGTCTTACTATTCTGCCTAT	514	Fritsche *et al*., 2008
	R : TCTTCCATTCCCTTCTCCTTT		
rmtD	F : ACGTGCGCCTCCATCCATTCG	338	Fritsche *et al*., 2008
	R : GCGTGCGGGCTGAATCCTGTC		
rmtA	F : TACGGCGGATTTTAGATCAGG	626	Fritsche *et al*., 2008
	R : TTCAAATTCATCAGGCAGTGC		
npmA	F : TGGGTACTGGAGACGGTAGAA	405	Fritsche *et al*., 2008
	R : CGCTCAAAAAATAGGCCTTAC		
qnrA	F : GATTTCTCACGCCAG GAT TT	578	Li, 2011
	R : GTT GCC AGG CAC AGA TCT TGA		
qnrB	F : ATA TGG CTC TGG CAC TCG TTG	415	Li, 2011
	R : TCCCACAGCTCACACTTTTCC		
qnrS	F : GATCTCACCTTCACCGCTTGC	521	Li, 2011
	R : TCGACTTTGCGGGGATCTAAA		
qnrC	F : GGGTTGTACATTTATTGAATCG	307	Li, 2011
	R : CACCTACCCATTTATTTTCA		
aac(6')-Ib	F : TTGCGATGCTCTATGGGTGGCTA	483	Park *et al*., 2006
	R : GCTCGAATGCCTGGCGTGTTT		

Random amplification of polymorphic DNA (RAPD): Seventeen distinct RAPD profiles were detected (Fig. 2). One profile (RAPD-A) was predominant (composed of 9 isolates). Six isolates were typed as RAPD-B; type RAPD-C and D contained 3 isolates each and 2 isolates were classified as type RAPD-E. The remaining 12 isolates were identified as 12 distinct types (1 isolate per type).

Proteus mirabilis PFGE analysis: Thirty-five *P. mirabilis* isolates were divided into 9 clusters by PFGE, revealing greater diversity than initially detected using RAPD. From the 9 clusters detected, there were 3 with 3 isolates and 6 with 2 isolates. The remaining 14 isolates were considered unrelated. Isolates from clusters were considered either to be the same strain (all clusters containing 2 isolates and 2 clusters of 3 isolates) or were closely related (2 clusters of 3 isolates) (Fig. 3).

Antimicrobial susceptibility testing: All *P. mirabilis* isolates were susceptible to cefepime, cefoxitin and cefotaxime/clavulanic, whereas 25 (71%) isolates were susceptible to cefotaxime and ceftriaxone (Table 3). Furthermore, most were resistant to ampicillin (71%), mezlocillin (60%) or trimethoprim/sulfamethoxazole (69%), whereas, resistance to amikacin (20%), ampicillin/ sulbactam (23%), piperacillin (43%), gentamicin (46%), tobramycin (51%), chloramphenicol (31%), ciprofloxacin (49%) and norfloxacin (37%) was relatively common. Two (6%) isolates were resistant to aztreonam but none was resistant to imipenem (Table 3).

Table 3: Antimicrobial resistance of 35 *Proteus mirabilis* isolated from dogs with diarrhea in Beijing

Antimicrobial	ESBL (n=13) (%)	non-ESBL (n=22) (%)	Total (n=35) (%)
Ampicillin	12 (92)[a]	13 (59)[b]	25 (71)
Ampicillin/sulbactam	4 (30)	4 (18)	8 (23)
Aztreonam	1 (8)	1 (5)	2 (6)
Piperacillin	9 (69)[a]	6 (27)[b]	15 (43)
Mezlocillin	12 (92)[a]	9 (41)[b]	21 (60)
Imipenem	0	0	0
Cefoxitin	0	0	0
Ceftriaxone	9 (69)[a]	4 (18)[b]	13 (37)
Cefotaxime	8 (62)[a]	5 (23)[b]	13 (37)
Cefotaxime/clavulanic	0	0	0
Amikacin	5 (38)	2 (9)	7 (20)
Gentamicin	6 (46)	10 (45)	16 (46)
Tobramycin	7 (54)	11 (50)	18 (51)
Tetracycline	13 (100)	22 (100)	35 (100)
Chloramphenicol	8 (62)[a]	3 (14)[b]	11 (31)
Trimethoprim/sulfamethoxazole	7 (54)	17 (77)	24 (69)
Ciprofloxacin	8 (62)	9 (41)	17 (49)
Norfloxacin	8 (62)[a]	5 (23)[b]	13 (37)

[a,b]Within a row, percentages without a common letter differed (P≤0.05) between ESBL-producing and non-ESBL-producing isolates for the respective antimicrobial.

Table 4: Distribution of 13 ESBL-producing genotypes in *Proteus mirabilis* isolated from fecal samples of dogs with diarrhea in Beijing, China

ESBLs types	RAPD types (No. of isolates)				Total (No. of isolates)
	A	E	L	N	
TEM	7	0	0	0	7
TEM/CTX-M-1	2	0	0	0	2
TEM/OXA-1	0	2	1	1	4
Total	9	2	1	1	13

Table 5: Distribution of 16S rRNA methylases genes and plasmid-mediated quinolone resistance genes in 35 *Proteus mirabilis* isolated from fecal samples of dogs with diarrhea in Beijing, China

Function	Genes	Isolates with detected genes	
		No.	%
16S rRNA methylases	*rmt*A	0	0
	*rmt*B	18	51
	*rmt*C	0	0
	*rmt*D	0	0
	*npm*A	0	0
	*arm*A	0	0
Plasmid-mediated quinolone resistance	*qnr*A	0	0
	*qnr*B	5	14
	*qnr*C	0	0
	*qnr*S	0	0
	aac(6')-Ib	9	26

Thirteen (37%) of 35 *P. mirabilis* isolates were ESBLs-producers. These isolates had decreased susceptibilities to ampicillin, piperacillin, mezlocillin, cefotaxime, ceftriaxone, chloramphenicol and norfloxacin (P<0.05; Table 3).

Detection of potential ESBLs genes: Among the 13 *P. mirabilis* isolates producing ESBLs, all harbored the *bla*$_{TEM}$ gene. Six isolates carried more than 1 ESBLs gene (Table 4). Seven of 9 RAPD-A isolates harbored only the *bla*$_{TEM}$ gene whereas the remaining 2 harbored both *bla*$_{TEM}$ and *bla*$_{CTX-M-1}$. Isolates with both *bla*$_{TEM}$ and *bla*$_{OXA-1}$ were detected belonged to RAPD-E (n=2), RAPD-L and RAPD-N types (Table 4).

Detection of genes encoding 16S rRNA methylases: The *rmtB* gene was detected in 18 (51%) isolates whereas *armA*, *rmtA*, *rmtC*, *rmtD* and *npmA* were not detected in the present study (Table 5).

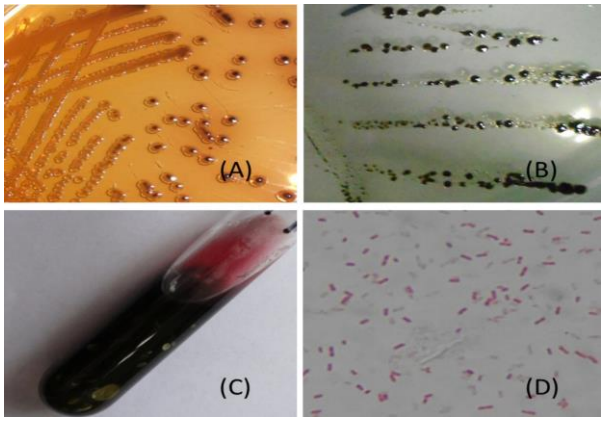

Fig. 1: Bacterial morphologies of *Proteus mirabilis.* (A) Growing on SS agar plate. (B) Growing on HE agar plate. (C) Growing in ferric trisaccharide semisolid medium. (D) Gram-stained preparation observed under an optical microscope (1000 x) (D).

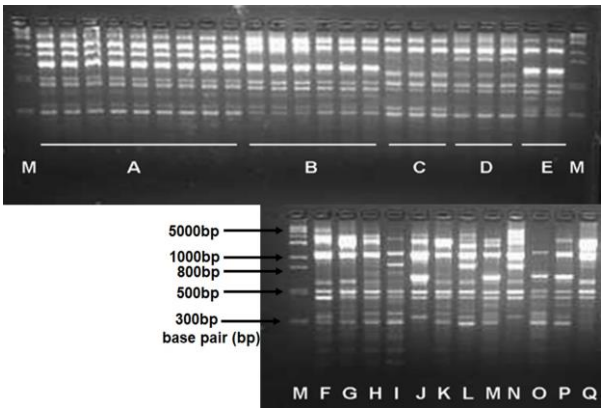

Fig. 2: Molecular typing of 35 *Proteus mirabilis* by RAPD. Isolates were divided into 17 RAPD types (A-Q), with a predominance of type A. Trans-5000 was applied as standard molecular size marker (Trans-Gen).

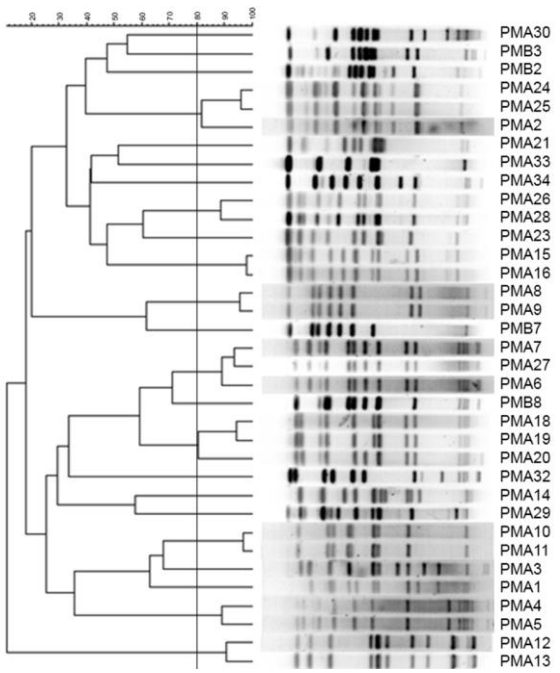

Fig. 3: Analysis of Pulsed-Field Gel Electrophoresis (PFGE) patterns obtained from the relevant *Proteus mirabilis* isolates. PFGE profile were compared by BioNumerics V 5.1 software**.** In this experiment, a global reference strain *Salmonella enterica* serotype Braenderup strain H9812, *Xbal* digestion, was used as the molecular marker (Marker) (Hunter *et al*., 2005).

Detection of plasmid-mediated quinolone resistance genes: Plasmid-mediated quinolone resistance (PMQR) genes *qnrB* and *aac(6')-Ib* were detected in 5 (14%) and 9 (26%) isolates, respectively. No other PMQR gene was detected. All positive isolates were resistant to, at least 1 tested quinolone (norfloxacin and ciprofloxacin) (Table 5).

DISCUSSION

To the best of our knowledge, this was the first report of the prevalence, antimicrobial susceptibility and genomic diversity of *P. mirabilis* isolated from canine fecal samples in China. Prevalence of *P. mirabilis* in feces from hospitalized diarrheic dogs in Beijing was estimated to be 28%. In the current study, despite the great diversity in *P. mirabilis* isolated, clustering of isolates was present using either RAPD or PFGE results. Five RAPD types (A, B, C, D and E) containing >1 isolate and indistinguishable isolates were present; consequently, we speculate that there is a common source of *P. mirabilis* in dogs with diarrhea (e. g. some RAPD types commonly isolated from UTIs and, therefore, being more prevalent in fecal content). Our data also demonstrated that 1 predominant type (RAPD-A) existed among tested isolates. Furthermore, potential ESBLs genes were identified in all type A isolates.

Antimicrobial resistance (AMR) in *P. mirabilis* isolated from dogs has been reported (Zhang *et al*., 2018). Broad-spectrum antimicrobials are frequently prescribed for treating bacterial infections in small animals. Overall prevalence of antimicrobial resistance in *P. mirabilis* isolated from dogs seemed higher than reported for human isolates (Siebor and Neuwirth, 2013). Prevalence of resistance to ampicillin (71%) was higher than the reported (Harada *et al*., 2014), probably due to presence of TEM penicillinases (Hordijk *et al*., 2013). The high prevalence of resistance to quinolone, trimethoprim/ sulfamethoxazole and aminoglycosides limits therapeutic options for infections caused by *P. mirabilis*, as these classes of antimicrobials are frequently used to treat dogs with infections caused by these bacteria (Wong *et al*., 2015).

ESBLs lead to extensive beta-lactam resistance, undermining efficacious therapeutic options for treating bacterial infections (Schultz *et al*., 2017). AMR rates differed between ESBL-positive and negative isolates. Among the non-beta-lactam antimicrobials, ESBL isolates also had higher resistance rates to chloramphenicol and some fluoroquinolones. The exact mechanism of increased resistance to non-beta-lactam antimicrobials in ESBL-producing strains remains unknown.

Proteus mirabilis is amongst the most common cephalosporin-resistant bacterial isolates from dogs (Fritsche *et al*., 2008). In our study, the bla_{TEM} gene was detected in all ESBL-positive isolates. TEM-type ESBLs are among the most prevalent in *P. mirabilis* isolated from hospitalized patients (Ahn *et al*., 2017; Rajivgandhi *et al*., 2018). Despite being commonly present in members of the Enterobacteriaceae family (Alonso *et al*., 2017), bla_{SHV} genes were not detected in any strain, corroborating recent results (Zhang *et al*., 2018). Prevalence of *OXA-1* positive isolates in this study was much higher than in a French report (Bonnet *et al*., 2002).

Aminoglycosides bind irreversibly to the 30S small subunit of bacterial ribosomes, inhibiting mRNA transcription and protein synthesis. Resistance against amikacin (20%) was lower than that of gentamicin (46%) and tobramycin (51%), consistent with previous reports (Wieczorek *et al*., 2008). The *rmtB* gene was detected in 18 *P. mirabilis* isolates; half of these isolates were resistant to all aminoglycosides evaluated (gentamicin, kanamycin, tobramycin, streptomycin, amikacin). Perhaps high resistance to aminoglycosides was due to presence of the *rmtB* gene (Fritsche *et al*., 2008).

qnrB and *aac(6')-Ib* are important genes associated with decreased susceptibility to fluoroquinolones. In our study, presence of PMQR genes was always followed by resistance to fluoroquinolones. It is well-established that resistance against fluoroquinolones may also occur due to mutations in the quinolone-resistance determining region of specific genes (de Jong *et al*., 2018); therefore, mechanisms other than those screened could have been present for fluoroquinolone-resistant *P. mirabilis* isolates where no resistance genes were detected.

A previous study reported that companion animals and humans could be infected with closely related *P. mirabilis* strains, suggesting a potential role for companion animals as reservoirs of *P. mirabilis* to humans (Marques *et al*., 2019). Therefore, higher frequency of elimination of *P. mirabilis* to the environment through diarrhea in dogs, combined with high antimicrobial resistance of *P. mirabilis* in this study, represented a potential threat for human health.

This study had some limitations. Although *Proteus* spp. can be recovered from healthy individuals, they are more frequently isolated from feces of patients suffering from diarrhea. Whereas it is tempting to assume that *P. mirabilis* were the causative agents of diarrhea in studied dogs, especially due to their apparent high prevalence, there were not definitively implicated as the cause. Presence of enteropathogenic bacteria causing any intestinal disorder in dogs is clouded by presence of normal indigenous flora. Therefore, in the present study, no causal association can be established between presence of *P. mirabilis* and diarrhea in dogs. Additionally, as ESBL genes such as bla_{TEM} were not sequenced, we cannot be sure whether variants detected were ESBL-associated genes or not.

Conclusions: *P. mirabilis* was commonly isolated from diarrheic dogs hospitalized in Beijing. A predominant RAPD type (A) was observed. Clustering of isolates was relatively common; several isolates were either indistinguishable or closely related. Isolates had high resistance to a majority of common antimicrobials. bla_{TEM} gene was detected in all ESBL-producing isolates.

Acknowledgments: This research was financially supported by the National Natural Science Foundation of China (No. 31772813, 31572587), the High-end Foreign Experts Recruitment Program (No. GDT20171100013), and the National Key R&D Project (No. 2016YFD0501203). The authors thank Dr. John Kastelic (University of Calgary, Calgary, AB, Canada) for editing the manuscript.

Authors contribution: PH and JG conceived and designed the study. PH, LL and SM executed the experiment. PH, ML, SW, DN, NS, HB and BH analyzed the data. We confirm that all authors have interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

REFERENCES

Adams-Sapper S, Sergeevna-Selezneva J, Tartof S, *et al*., 2012. Globally dispersed mobile drug-resistance genes in Gram-negative bacterial isolates from patients with bloodstream infections in a US urban general hospital. J Med Microbiol 61:968-74.

Ahn JY, Ann HW, Jeon Y, *et al*., 2017. The impact of production of extended-spectrum β-lactamases on the 28-day mortality rate of patients with *Proteus mirabilis* bacteremia in Korea. BMC Infect Dis 17:327.

Alonso CA, Michael GB, Li J, *et al*., 2017. Analysis of blaSHV-12-carrying *Escherichia coli* clones and plasmids from human, animal and food sources. J Antimicrob Chemother 72:1589-96.

Bonnet R, Marchandin H, Chanal C, *et al*., 2002. Chromosome-encoded class d-lactamase OXA-23 in *Proteus mirabilis*. Antimicrob Agents Chemother 46:2004-6.

Clinical and Laboratory Standards Institute, 2015. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. CLSI document VET01S, 3rd ed. Wayne, PE, USA.

de Jong A, Muggeo A, El Garch F, *et al*., 2018. Characterization of quinolone resistance mechanisms in *Enterobacteriaceae* isolated from companion animals in Europe (ComPath II study). Vet Microbiol 216:159-67.

Drzewiecka D, 2016. Significance and roles of *Proteus* spp. bacteria in natural environments. Microb Ecol 72:741-58.

Fritsche TR, Castanheira M, Miller GH, *et al*., 2008. Detection of methyltransferases conferring high-level resistance to aminoglycosides in enterobacteriaceae from Europe, North America and Latin America. Antimicrob Agents Chemother 52:1843-5.

Harada K, Niina A, Shimizu T, *et al*., 2014. Phenotypic and molecular characterization of antimicrobial resistance in *Proteus mirabilis* isolates from dogs. J Med Microbiol 63:1561-7.

Hordijk J, Schoormans A, Kwakernaak M, *et al*., 2013. High prevalence of fecal carriage of extended spectrum β-lactamase/AmpC-producing *Enterobacteriaceae* in cats and dogs. Front Microbiol 4:242.

Hu HM, 2007. Detection and genotypes distribution of extended-spectrum β-lactamases in *Proteus* spp. Zhejiang University, Master's thesis, Zhejiang, China.

Hunter SB, Vauterin P, Lambert-Fair MA, *et al*., 2005. Establishment of a universal size standard strain for use with the pulsenet standardized pulsed-field gel electrophoresis protocols: Converting the national databases to the new size standard. J Clin Microbiol 43:1045-50.

Jacobsen SM and Shirtliff ME, 2011. *Proteus mirabilis* biofilms and catheter-associated urinary tract infections. Virulence 2:460-5.

Jay-Russell MT, Hake AF, Bengson Y, *et al*., 2014. Prevalence and characterization of *Escherichia coli* and *Salmonella* strains isolated from stray dog and coyote feces in a major leafy greens production region at the United States-Mexico border. PLoS One 9:e113433.

Li DX, 2011. Detection and diffusion mechanism of 16S rRNA methylase gene from *Proteus mirabilis* and *Salmonella* species. Henan Agricultural University, Masters thesis, Henan, China.

Lin MF, Liou ML, Kuo CH, *et al*., 2019. Antimicrobial susceptibility and molecular epidemiology of *Proteus mirabilis* isolates from three hospitals in northern Taiwan. Microb Drug Resist. doi: 10.1089/mdr.2019.0066.

Luo ZF, Zhang WY and Yang DS, 2008. Detection of *Proteus mirabilis* in animal-derived food by polymerase chain reaction. Fujian Anim Sci Vet Med 30:1-3.

Kim SH, Wei CI and An H, 2005. Molecular characterization of multidrug-resistant *Proteus mirabilis* isolates from retail meat products. J Food Prot 68:408-13.

Marques C, Belas A, Aboim C, *et al*., 2019. Clonal relatedness of *Proteus mirabilis* strains causing urinary tract infections in companion animals and humans. Vet Microbiol 228:77-82.

Michelim L, Muller G, Zacaria J, *et al*., 2008. Comparison of PCR-based molecular markers for the characterization of *Proteus mirabilis* clinical isolates. Braz J Infect Dis 12:423-9.

Park CH, Robicsek A, Jacoby GA, *et al*., 2006. Prevalence in the United States of aac(6')-Ib-cr encoding a ciprofloxacin-modifying enzyme. Antimicrob Agents Chemother 50:3953-5.

Schaffer JN and Pearson MM, 2015. *Proteus mirabilis* and urinary tract infections. Microbiol Spectr 3:1-39.

Rajivgandhi G, Maruthupandy M and Manoharan N, 2018. Detection of TEM and CTX-M genes from ciprofloxacin resistant *Proteus mirabilis* and *Escherichia coli* isolated on urinary tract infections (UTIs). Microb Pathog 121:123-30.

Schultz E, Cloeckaert A, Doublet B, *et al*., 2017. Detection of SGI1/PGI1 elements and resistance to extended-spectrum cephalosporins in proteae of animal origin in France. Front Microbiol 8:32.

Siebor E and Neuwirth C, 2013. Emergence of Salmonella genomic island 1 (SGI1) among *Proteus mirabilis* clinical isolates in Dijon, France. J Antimicrob Chemother 68:1750-6.

Tenover FC, Arbeit RD, Goering RV, *et al*., 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 33:2233-9.

Wang JT, Chen PC, Chang SC, *et al*., 2014. Antimicrobial susceptibilities of *Proteus mirabilis*: a longitudinal nationwide study from the Taiwan surveillance of antimicrobial resistance (TSAR) program. BMC Infect Dis 14:486.

Wieczorek P, Sacha P, Hauschild T, *et al*., 2008. The aac(6')Ib gene in *Proteus mirabilis* strains resistant to aminoglycosides. Folia Histochem Cytobiol 46:531-3.

Wong C, Epstein SE and Westropp JL, 2015. Antimicrobial susceptibility patterns in urinary tract infections in dogs (2010-2013). J Vet Intern Med 29:1045-52.

Yazdi M, Bouzari M and Ghaemi EA, 2018. Isolation and characterization of a lytic bacteriophage (vB_PmiS-TH) and its application in combination with ampicillin against planktonic and biofilm forms of *Proteus mirabilis* isolated from urinary tract infection. J Mol Microbiol Biotechnol 28:37-46.

Zhang PLC, Shen X, Chalmers G, *et al*., 2018. Prevalence and mechanisms of extended-spectrum cephalosporin resistance in clinical and fecal *Enterobacteriaceae* isolates from dogs in Ontario, Canada. Vet Microbiol 213:82-8.