Short Paper

Comparison of virulence genes in *Proteus* species isolated from human and pet turtle

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Summary

The current study was aimed to investigate the prevalence of *ureC*, *rsbA*, *mrpA* and *mrpA* virulence genes using polymerase chain reaction (PCR) in *Proteus* spp. isolated from 5 commercially popular species of pet turtles and comparison of the *mrpA* gene sequences of *Proteus mirabilis* isolates with human clinical isolates. A total of 24 isolates in pet turtles were identified, comprised of *P. mirabilis* (15), *Proteus vulgaris* (7) and *Proteus hauseri* (2). The prevalence of *ureC*, *rsbA*, *mrpA* and *mrpA* genes among all identified *Proteus* spp. isolates were 91.7%, 50%, 45.8% and 45.8%, respectively. The average percentage similarities of *mrpA* gene sequence of pet turtle *P. mirabilis* isolates to human urinary and respiratory isolates were 96.35% and 94.85%, respectively. The prevalence of virulence genes and high similarity of *mrpA* gene sequences between pet turtles and human *P. mirabilis* isolates revealed that though pet turtles are healthy, these animals may pose a potential risk of urinary and respiratory infections to humans.

Key words: *mrpA* gene, Pet turtles, *Proteus* spp., Virulence genes

Introduction

The genus *Proteus* is a Gram-negative bacillus that belongs to the Enterobacteriaceae family. Members of the genus *Proteus* are widespread in the environment and the gastrointestinal tract of human and animals (Hegazy, 2016).

*Proteus* is known as a nosocomial, opportunistic pathogen and is more common in community-acquired infections (Omoriyia and Evangelista, 2014). *Proteus mirabilis* and *P. vulgaris* have been reported to cause wound infections, respiratory tract infections and both community-acquired and catheter-associated urinary tract infections (UTI) (Li and Mobley, 2002; Trivedi et al., 2015). In addition, Hordijk et al. (2013) have reported urinary and kidney infections of companion animals, diarrhea in cats and dogs.

Virulence of the *Proteus* spp. is caused by several virulent factors and these virulent factors are regulated by virulent genes encoded in operons (Manos and Belas, 2006). This study focused on *zapA*, *rsbA*, *mrpA* and *ureC* genes. Since they have been identified to code most important virulent factors and have been found to be more common in previous studies (Abbas et al., 2015; Alsherees et al., 2016). One of the prominent features of *Proteus* spp. is the ability to swarm on solid surfaces. Even though several genes are associated with the swarming phenomenon as *cheW*, *gidA* and *cldA* genes, the *rsbA* gene is important for the swarming regulation (Rather, 2005).

Urease is the most important enzyme for kidney and bladder stone formation in *Proteus* infection. The *ureA*, *ureB*, *ureC*, *ureD*, *ureE*, *ureF*, *ureG* and *ureR* genes on *ure* operon are responsible for the production process of urease enzyme and previous study pointed out *ureC* as a major gene, causative for urease production (Li and Mobley, 2002). The *zap* operon encoded by *zapA*, *zapB*, *zapC* and *zapD* genes is important for the production of protease, especially *zapA* for regulating IgA protease expression during the differentiation of swarmers cells to swarmer cells (Walker et al., 1999).

A variety of fimbriae have been detected in *P. mirabilis*. The vital type is MR/P fimbria encoded by *mrpA*, *mrpB*, *mrpC*, *mrpD*, *mrpE*, *mrpF*, *mrpG* and *mrpI* genes. The *mrpA* gene is significantly important to the pathogenicity, since it contributes numerous virulent factors such as adherence of bacteria to the epithelial tissue, biofilm formation, and swarming phenomenon (Rocha et al., 2007).

According to the comparison of genetic characteristics among bacterial isolates of human and animal origin, the issue of possible transmission risk to human has been discussed previously (Barbour et al., 2012). Nucleotide sequences of a few genes of human...
isolated *P. mirabilis* were compared with chicken and dog isolates with regard to fimbriae (Barbour et al., 2012; Harada et al., 2014).

Nowadays, pet turtles are known as a potential risk in public health, and *Proteus* spp. cause anorexia, pneumonia, depression, and the death of pet turtles (Henriksen, 1972). Therefore, the objectives of this study were to investigate *mpmA*, *zapA*, *rsbA* and *ureC* genes in *Proteus* spp. isolated from pet turtles and to compare the acquired *mpmA* sequences of *P. mirabilis* with human clinical isolates.

**Materials and Methods**

Fifty-two pet turtles including 2 African side-neck turtles (*Pelusios castaneus*), 31 Chinese stripe-necked turtles (*Ocadia sinensis*), 10 river cooters (*Pseudemys concinna concinna*), 3 Western painted turtles (*Chrysemys picta belli*) and 6 yellow-bellied sliders (*Trachemys scripta scripta*) were purchased from pet shops and online markets. The turtles were under 4 weeks of age. The turtles were raised under laboratory conditions following the general husbandry method (Bluvias and Eckert, 2010).

Isolation and identification of *Proteus* spp. were performed according to standard procedure (Senior, 1997; Back et al., 2016). PCR amplification of *mpmA*, *zapA*, *rsbA* and *ureC* genes was performed using specific primers and PCR conditions given in Table 1.

Randomly selected five *mpmA* amplicons of *P. mirabilis* were purified using Expin™ PCR SV kit (GeneAll®, Korea) and sent to Cosmogenetech Co. Ltd., Daejeon, Korea for direct sequencing. The nucleotide sequences of the *mpmA* gene from turtles were compared with the sequences of the *mpmA* gene from human isolates (respiratory and urinary) previously reported by Barbour et al. (2012) and using BLAST option of NCBI (Basic Local Alignment Search Tool, BLAST v. 2.2.15, www.ncbi.nlm.nih.gov).

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

**Results**

Twenty-four isolates were positive in biochemical tests and 16S rRNA sequencing could identify them up to species level. Out of 24 bacterial isolates, 2, 7 and 15 isolates were identified as *P. hauseri*, *P. vulgaris* and *P. mirabilis*, respectively. The population of isolated bacteria predominantly consisted of *P. mirabilis* (Table 2).

The *UreC* was the most prevalent gene and was identified in twenty-two isolates (91.7%). However, the *ureC* gene was detected only in *P. mirabilis* and *P. vulgaris* with 100% detection rate in both species (Table

### Table 1: Description of gene targets and the corresponding primers used for the specific virulent genes in PCR assay

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer type</th>
<th>Primer sequence 5'-3'</th>
<th>Size bp</th>
<th>T (°C)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>mpmA</em></td>
<td><em>mpmAF</em></td>
<td>ACACCTGGCCCATATGGAATGATACTGTTCA</td>
<td>550</td>
<td>40°C</td>
<td>Barbour et al. (2012)</td>
</tr>
<tr>
<td></td>
<td><em>mpmAR</em></td>
<td>AAGTGATGAAGCTTATGCTGATGGATGATGAGTAAAGTCC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>rsbA</em></td>
<td><em>rsbAF</em></td>
<td>TTGAGGAGCCGAGTACGACC</td>
<td>467</td>
<td>58°C</td>
<td>Abbas et al. (2015)</td>
</tr>
<tr>
<td></td>
<td><em>rsbAR</em></td>
<td>ACTCTGGCTCTCGTTGGA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>ureC</em></td>
<td><em>ureCF</em></td>
<td>GTATTCAATGATGGTATGG</td>
<td>317</td>
<td>56.2°C</td>
<td>Ali and Yousif (2015)</td>
</tr>
<tr>
<td></td>
<td><em>ureCR</em></td>
<td>ATAAAGGTTGTTACGCCAGA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Zap</em></td>
<td><em>ZapAF</em></td>
<td>ACGGGCAGGAAACATATAGCCC</td>
<td>540</td>
<td>59°C</td>
<td>Ali and Yousif (2015)</td>
</tr>
<tr>
<td></td>
<td><em>ZapAR</em></td>
<td>GCGACTATCTTCCGCTAAATCA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* Annealing temperature

### Table 2: Distribution of *Proteus* spp. isolated from pet turtles

<table>
<thead>
<tr>
<th>Gene</th>
<th>Number (%) of positive strains for genes</th>
<th>Total positive strains (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. mirabilis</em> (n=15)</td>
<td><em>P. vulgaris</em> (n=7)</td>
</tr>
<tr>
<td><em>ureC</em></td>
<td>15 (100)</td>
<td>7 (100)</td>
</tr>
<tr>
<td><em>rsbA</em></td>
<td>12 (80)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>zapA</em></td>
<td>11 (73.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>mpmA</em></td>
<td>11 (73.3)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

### Table 3: Distribution of virulent genes of *Proteus* spp. isolated from pet turtles

<table>
<thead>
<tr>
<th>Turtle</th>
<th>Number (%) of isolates</th>
<th>Isolation rate of <em>Proteus</em> spp. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. mirabilis</em></td>
<td><em>P. vulgaris</em></td>
<td><em>P. hauseri</em></td>
</tr>
<tr>
<td>Chinese stripe-necked turtle (n=31)</td>
<td>7 (22.6)</td>
<td>4 (12.9)</td>
</tr>
<tr>
<td>River cooter (n=10)</td>
<td>6 (60)</td>
<td>2 (20)</td>
</tr>
<tr>
<td>Yellow-bellied slider (n=6)</td>
<td>1 (16.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Western painted turtle (n=3)</td>
<td>1 (33.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>African side-neck turtle (n=2)</td>
<td>0 (0)</td>
<td>1 (50)</td>
</tr>
<tr>
<td>Total (n=52)</td>
<td>15 (28.8)</td>
<td>7 (13.5)</td>
</tr>
</tbody>
</table>
Among four virulent genes, rsbA was detected at the ratio of 50% (12/24) while zapA and mrpA exhibited similar percentage, 45.8% (11/24). 80% of rsbA, 73.3% of zapA, and 73.3% of mrpA were observed in P. mirabilis (Fig. 1). In P. vulgaris ureC was the only gene detected and none of the genes were identified in P. hauseri isolates.

Mean similarity percentages with regard to mrpA gene of pet turtles and four human urinary P. mirabilis isolates were 96.2%, 96.4%, 97.0%, and 95.8%, respectively. Besides, human respiratory and turtle isolates showed 94.4%, 95.4%, 94.6%, and 95.0% of similarity percentages. Mean of means was calculated using similarity percentages and it was 96.4% in urinary isolates and 94.9% in respiratory isolates (Table 4).

**Discussion**

Proteus spp. was detected in many wild and domestic animals particularly with no clinical signs (Drzewiecka, 2016). Since Proteus spp. is an opportunistic pathogen, even healthy turtles can be asymptomatic carriers of the pathogen (Hossain et al., 2016). In the present study, P. mirabilis was the predominant species followed by P. vulgaris as 62.5% (15/24) of P. mirabilis, 29.17% (7/24) of P. vulgaris and 8.34% (2/24) of P. hauseri. Dalia (2015) has reported the same results with clinical isolates.

The current study was mainly focused on the detection of virulence genes. Several virulence determinants contribute to the pathogenicity of Proteus spp. (Schaffer and Pearson, 2015). The ureC gene is responsible for the elevation of pH of urine and resulting in stone formation (Mohammed et al., 2014). In this study, ureC amplification of P. mirabilis and P. vulgaris was 100%, which reveals a higher frequency of ureC compared with the rest of the genes. It indicates the crucial role of ureC for the virulence of the Proteus spp.
Regarding ureC gene, these results are in agreement with a previous study, indicating the presence of ureC approximately 96.6% among isolates obtained from patients with UTI (Ali and Yousif, 2015).

The rsbA gene could not be amplified in P. vulgaris and P. hauseri. However, the majority of positive strains were P. mirabilis and it is in line with the previous studies (Rather, 2005; Abbas et al., 2015). In the current study, a phenotypic character of swarming, the bull’s-eye ring was exhibited by all the isolates. Nevertheless, swarming regulated genes are not necessarily required for swarming, for the reason that so many genes and operons are involved in the process (Pearson et al., 2010).

The zapA gene which is coding for protease enzyme production and mrpA gene which is mainly important for fimbreriae gene were identified in similar number of isolates 11 (45.8%). The zapA and mrpA could not be detected in P. vulgaris and P. hauseri isolates. Nevertheless, the frequency of both genes in P. mirabilis was 73.3% in disagreement with previous studies which were 30% (Alsherees et al., 2016) and 100% (Ali and Yousif, 2015) in clinical P. mirabilis isolates. Although the prevalence of mrpA gene in previous studies was small in percentage values, the most important gene among tested gene is mrpA due to its contribution to several virulent factors (Abbas et al., 2015). Therefore, mrpA was the selected gene for comparison of gene sequences.

Furthermore, comparison of mrpA amplicon sequences of pet turtles to human isolates revealed similarity percentages. Fimbriae are imperative for several virulent factors of Proteus species. Among 6 fimbreriae types and 17 fimbrial operons, MR/Pis the most important fimbria (Różalski et al., 2012). This type of fimbria is encoded by mrp operon containing 10 genes located on the bacterial chromosome. The main structural subunit of these fimbriae is the MrpA protein. Though other fimbrияe are present only in P. mirabilis, the mrp operon was shown not only in P. mirabilis strains but also in other species of the Proteus genus. In our results, comparison of pet turtles to human urinary isolates, using mrpA gene of P. mirabilis represents 96.40% of mean similarity and pet turtles to human respiratory isolates using the same gene of P. mirabilis represents 94.90% of mean similarity.

Previous studies proved that direct contact even with healthy turtles can transmit diseases to humans and if people, especially immune suppressed adults and children, have direct physical contact with turtles and aquarium water, there might be a potential risk to transmit pathogens and to cause urinary infections (Mermin et al., 2004; Hidalgo-Vila et al., 2007).

In conclusion, the high prevalence of Proteus species, especially P. mirabilis in pet turtles, and their similarity of virulence gene characteristics with human clinical isolates could be emphasized. Thus the potential health risk of infection of Proteus spp. from pet turtles should not be underestimated. Expanding this research further with more virulent genes could be important for a better understanding of the pathogenicity of Proteus spp. harbored by pet turtles. According to our knowledge, this is the first report designed to detect zapA, rsbA, mrpA and ureC genes in Proteus isolates of pet turtles.

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Conflict of interest

The authors declare no conflict of interests.

References

Hossain, S; Wimalasena, SHMP and Heo, GJ (2016). Virulence factors and antimicrobial resistance pattern of...


