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SHORT COMMUNICATION

Demonstration of virulent genes within *Listeria* and *Klebsiella* isolates contaminating the export quality frozen shrimps

Rashed Noor · Md. Faqrul Hasan · Md. Sakil Munna · M. Majibur Rahman

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Abstract In the previously studied export quality shrimp samples, presence of a range of pathogenic bacteria including *Listeria* spp. and *Klebsiella* spp. were detected. The presence of *eae* gene in *Escherichia coli*, *aero* specific gene in *Aeromonas* spp., and *sodB* gene in *Vibrio* spp. were observed which might confer the associated virulence. Present study further attempted to detect the existence of virulent genes in *Listeria* spp. and *Klebsiella* isolates from the same shrimp samples through the gene specific polymerase chain reaction (PCR). Visualization of bands of *iap* (457 bp) and *gyr*B2 (711 bp) genes after PCR amplification referred the presence of virulence strains of *Listeria* spp. and *Klebsiella* spp., respectively in the export quality shrimp samples. Together with the previous findings of virulence genes of several pathogenic bacteria, the outcomes of current study further conferred the possible detrimental impact of the export quality frozen shrimps as and when consumed.

Keywords Consumer health safety · Export quality frozen shrimps · *Listeria* spp. · *Klebsiella* spp. · *lap* · *gyr*B2

Findings

Although the export quality frozen shrimps cover the prime economic importance in Bangladesh, they are not unlikely to be microbiologically contaminated resulting in various food-borne disease outbreaks (Rahman et al. 2012; Noor et al. 2013; Hassan et al. 2013; Ahmed et al. 2013; World Health Organization 2008, 2013). Inappropriate conditions of transport, handling and processing may impart such problem associated with quality of shrimps (Noor et al. 2013; Hassan et al. 2013; Ahmed et al. 2013; Sultana et al. 2014; Ghalis et al. 2010; Rahman et al. 2012). Besides, the subsistence of virulent genes could be of clinical importance

R. Noor (✉) · Md. Sakil Munna
Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka 1217, Bangladesh
e-mail: noor.rashed@yahoo.com

Md. Sakil Munna
e-mail: sakilmunna@gmail.com

Md. Faqrul Hasan · M. Majibur Rahman
Department of Microbiology, University of Dhaka, Dhaka 1000, Bangladesh
e-mail: faqrulhasan10225@gmail.com

M. Majibur Rahman
e-mail: majibrahman@gmail.com

contributing to the pathogenesis of the contaminating bacteria (Hotopp 2011; Amin and Salem 2012; Yuan et al. 2013; Postollec et al. 2011; Nakamura et al. 2013).

Listeria monocytogenes is known to be an opportunistic foodborne pathogenic bacterium generally triggering listeriosis, and also may lead to the onset of meningitis, sepsis, etc. (Painter and Slutsker 2007; Scallan et al. 2011). Among several food borne complications like diarrhoea, cholera, salmonellosis, etc., commencement of listeriosis also emerged as a major food borne health threat round the globe (Ross 2014). *L. monocytogenes* have been vastly reported to contaminate raw and undercooked meats, raw vegetables and fruits, unpasteurized milk, soft cheeses, etc. (Senjuti et al. 2014; Tahera et al. 2014; Marjan et al. 2014; Sarker et al. 2013). The *iap* gene of *L. monocytogenes* has been reported to be essential for maintaining the cell viability (Krawczyk-Balska et al. 2005).

Besides *Listeria* spp., bacteria belonging to the genus *Klebsiella* are well known for the onset of common food borne diseases (Abdaslam et al. 2014). Seafoods have been found earlier to be contaminated by *Klebsiella* spp. at the processing stages, possibly due to the poor handling and hygiene maintenance (Hamdan et al. 2008). DNA gyrase in *Klebsiella* spp., encoded by *gyrB2* gene, is a prokaryotic type II topoisomerase which is required for DNA replication as well as to maintain the cell viability (Nitiss 2009; Gore et al. 2006).

The molecular techniques have been proven to be promising in the disease diagnosis and estimating the prophylaxis in recent years for the purpose of detection of pathogens as well as the associated virulent genes (Liu et al. 2008). The polymerase chain reaction (PCR) has been reported to be the most useful and cost effective tool (Noor et al. 2014; Liu et al. 2008; Yu et al. 2007). In Bangladesh, the molecular aspect of pathogenic diagnosis of the export quality shrimps still remains scarce (Noor et al. 2014). In our recent study, the gene specific PCR method revealed the presence of *eae* gene in *E. coli*, *aero* specific gene in *Aeromonas*, *sodB* gene in *Vibrio* spp. (Noor et al. 2014). Besides, as stated earlier, some other genes from *Listeria* spp. and *Klebsiella* spp. could also be important for imparting the virulence of the respective bacteria as isolated in our previous study (Noor et al. 2014; Nawaz et al. 2012). Based on this rationale, current investigation further intended to detect the presence of virulent genes in the pathogenic isolates of *Listeria* spp. and *Klebsiella* spp.

Methods

As has been stated in our recent study (Noor et al. 2014), a total of 30 export quality frozen shrimp samples were collected and was processed for conducting the associated microbiological load estimation including *Listeria* spp. and *Klebsiella* spp. (Noor et al. 2014). To meet the objective of the current study, the genomic DNA from each of the two bacterial isolates was extracted through the modified boiling method and used for specific gene amplification (Noor et al. 2014). The primers used for *iap* gene (457 bp) in *Listeria* isolates were 5′-ATGTCATGGAATAA-3′ and 3′-GCTTTTCCAAGGTGTTTTT-5′; and for the amplification of *gyr*B2 gene (711 bp) within *Klebsiella* isolates, 5′-TCCGGCGGTCTGCACGGCGT-3′ and 3′-TTGTCCGGG TTGTACTCGTC-5′ (Liu et al. 2008; Yu et al. 2007). Amplification of *lap* gene through PCR was conducted employing the initial denaturation at 94 °C for 1 min followed by further denaturation at 92 °C for 1 min, primer annealing at 60 °C for 1 min and extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. Thirty (30) PCR cycles were executed in preface experiment within the exponential phase of amplification. Subsequently, amplification of *gyr*B2 was done with an initial denaturation at 94 °C for 4 min followed by the denaturation step at 94 °C for 1 min, primer annealing at 62 °C for 1–2 min and extension at 72 °C for 90 s followed by a terminating extension step at 72 °C for 10 min. PCR products were resolved through 1.2 % gel electrophoresis, stained with ethidium bromide, and visualized by UV trans-illuminator (Gel Doc, Bio-Rad Laboratories, In., USA).

Results and discussion

Although fish has been known to serve as the major nutrient supplements for human and other animals, the associated microbial spoilage of fish during the harvesting, handling, transportation, storage, and unhygienic maintenance is too common to affect the global food safety as well as the economy (Noor et al. 2013;

Acharjee et al. 2014). Considering the popularity for fish consumption together with the business perspectives, it is worth to control the microbiological quality of the fish to ensure the public health safety.

The bacterial bio-burden of *Listeria* spp. and *Klebsiella* spp. in the export quality frozen shrimp samples have already been stated in our recent study (Noor et al. 2014). In the present study, the gene specific PCR study revealed the presence of *Listeria* specific gene *iap* (encoding the invasion associated protein p60) in the tested *Listeria* isolates (as shown for two samples in Fig. 1). The isolates of *Klebsiella* spp. exhibited the presence of *gyr*B2 gene encoding DNA gyrase βII subunit (Fig. 2).

Our earlier microbiological and molecular studies on the export quality shrimps demonstrated a huge microbial contamination, possibly due to the lack of hygienic maintenance during the finished product processing and inappropriate storage (Rahman et al. 2012; Hassan et al. 2013; Ahmed et al. 2013; Sultana et al. 2014; Noor et al. 2014). The tested bacterial isolates were found almost to be drug-resistant (Noor et al. 2014). Also, our investigation on the propagation of the virulent genes (presence of *eae*, *sodB* and *aero* specific genes) within the export quality shrimps demonstrated the molecular basis of shrimp related fatality upon consumption (Noor et al. 2014). According to present study, export quality shrimps have been found to harbor the additional virulent genes in *Listeria* spp. and *Klebsiella* spp. which further added the new molecular insight into the current knowledge on the shrimp quality.

Considering the food safety as well as the public health significance, molecular study of the virulence traits of fish borne pathogens besides the pathogenic load is of significance in light of the knowledge on the harmful gene propagation among the fish. Besides bacterial burden, the successful detection of the virulent genes in fish may further forecast on the possible remedies with accuracy. Together with our recent findings (Noor et al. 2014), the results of the current study further revealed the existence of the virulent genes in the export quality

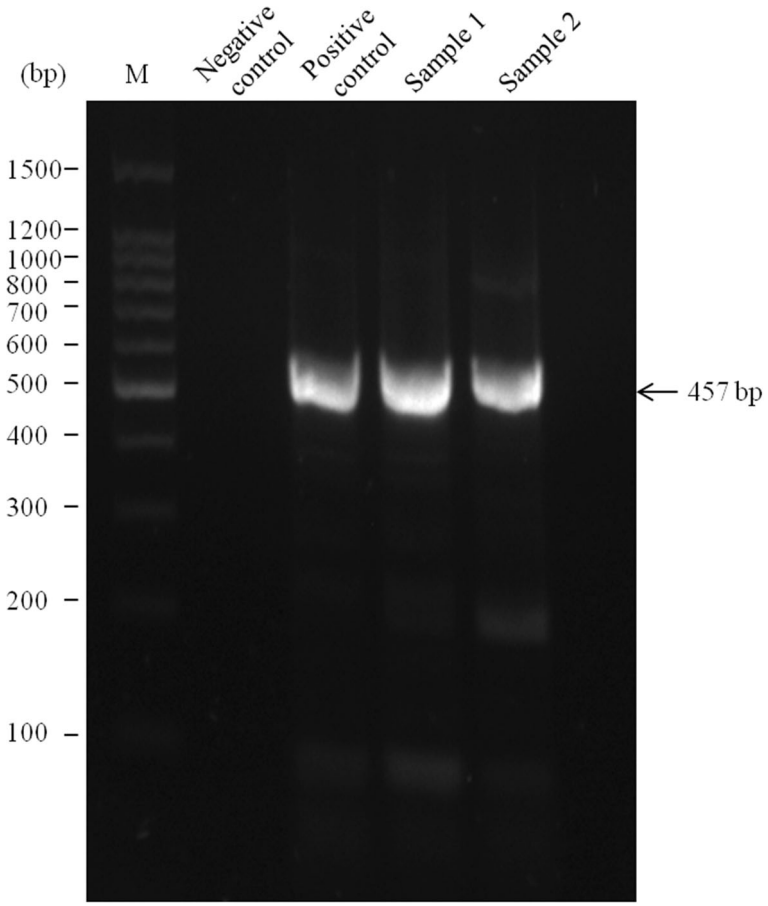

Fig. 1 Detection of the existence of *iap* gene in *Listeria* isolates. The PCR products of *iap* gene were resolved through 1.2 % agarose gel electrophoresis. As the positive control, the amplified DNA of *Listeria monocytogenes* (ATCC 19115) was used. *M* Marker

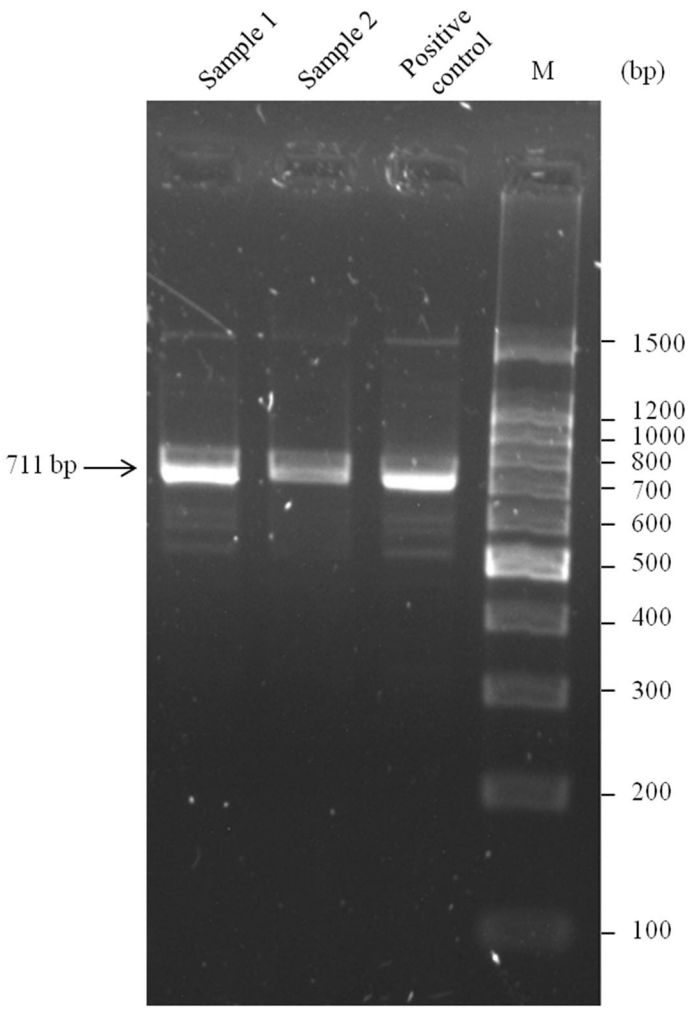

Fig. 2 Detection of *gyr*B2 gene within the *Klebsiella* isolates. The PCR products of *gyr*B2 gene were resolved through 1.2 % agarose gel electrophoresis. As the positive control, *Klebsiella pneumoniae* (ATCC 700603) was used. *M* Marker

shrimps contaminated with *Listeria* and *Klebsiella* isolates, thereby establishing the necessity of the routine molecular diagnosis for the sake of precise regulation of the microbiological quality of fish.

Author's contribution This work was carried out in collaboration between all authors. Author RN managed literature searches during preparation of the manuscript draft, analyzed the data, revised and approved the final manuscript. Author MFH conducted the experiments. Author MSM initially drafted the manuscript. Author MMR designed the study and supervised the related experiments.

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Conflict of interest The authors have declared that they have no competing interests.

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