Available online at http://www.journalijdr.com

ISSN: 2230-9926

International Journal of Development Research
Vol. 09, Issue, 06, pp. 28367-28369, June, 2019

RESEARCH ARTICLE OPEN ACCESS

MOLECULAR STUDY BASED ON *PROTEUS MIRABILIS* CONTAMINATION IN CHICKEN MEAT IN SELECTED DISTRICTS OF KERALA, INDIA

***Febi A. Bakkar and Zubair. M**

Department of Zoology, University of Calicut, Malappuram, Kerala, India – 673635

ARTICLE INFO

Article History:
Received 17th March, 2019
Received in revised form
19th April, 2019
Accepted 11th May, 2019
Published online 30th June, 2019

Key Words:
Proteus mirabilis, chicken meat, human Pathogen, molecular detection.

****Corresponding author:*** **Febi A. Bakkar**

ABSTRACT

Proteus mirabilis is known human pathogen as a common cause of human urinary tract infection (UTI), nosocomial infection, wound infection and a possible causative agent of outbreaks of gastroenteritis, resulting from the consumption of contaminated food. In this study, we have analyzed bacteria in the chicken meat in selected districts of Kerala, India, using 16S rRNA gene partial sequencing.. 50 Samples of raw chicken meat were collected from slaughterhouses and meat shops in the study area by simple random sampling method. Bacteriological analysis was carried out and the black colonies appeared on the plates were selected and drew out for further confirmation by PCR analysis. The PCR product was sequenced using Sanger's method. Then the trimmed forward and reverse sequences were assembled by using Clustal Omega and consensus sequence was taken for analysis by nucleotide BLAST programme. By examining 50 samples, it was confirmed that 1 sample from Kottapuram (Malappuram district) was contaminated by *P. mirabilis*. Maintaining a proper hygienic environment is an important step to avoid *P. mirabilis* related health hazards in humans or spreading to other birds. Microbial control in each and every stage of chicken production can control an outbreak of *P. mirabilis* contamination in chicken meat.

Citation: Febi A. Bakkar and Zubair, M. 2019. "Molecular study based on *Proteus mirabilis* contamination in chicken meat in selected districts of kerala, India", *International Journal of Development Research*, 09, (06), 28367-28369.

INTRODUCTION

The genus *Proteus* is a small gram-negative bacillus and a facultative anaerobe. It is widely distributed as saprophytes, living in soil, water, mammalian intestine, and in feces of humans and animals. These are opportunistic pathogens which are commonly responsible for infections in the urinary tract, septicemia, etc. The species of *Proteus* such as *Proteus mirabilis, Proteus vulgaris, and Proteus penneri* are the three human opportunistic pathogens (Guentzel, 1996). *Proteus mirabilis* is a normal flora seen in the human gastrointestinal tract. When this *P. mirabilis* enters the urinary tract, wounds, or the lungs it can become pathogenic. It usually causes urinary tract infections and nephrolithiasis. The most common infection from *P. mirabilis* occurs when the bacteria shift to the urinary bladder and urethra**.** One-hundred thousand Colony-forming units (CFU) per milliliter in the urine are typically indicative of a urinary tract infection (UTI). Pathogens that contaminate food materials can be effectively identified by the molecular level analysis. It is more efficient and hassle-free because of accurate identification by molecular level analysis.

Conventional methods such as culturing bacteria in selective medium and identified according to the biochemical or immunological characteristics are time-consuming and effortful. The present study focuses on the detection, isolation, and identification of bacterial strains from chicken meat collected from selected districts of Kerala, India. The bacteria isolated were identified by sequencing of the 16S rRNA gene. The primers used for sequencing are 27F and 1492R primer. Chicken meat can be contaminated by pathogenic bacteria like *P. mirabilis* which will affect the health of consumers as well as the person who handling it.

MATERIALS AND METHODS

Study area: The present study was carried out in different areas in selected districts of Kerala (Malappuram, Palakkad, Calicut, and Kannur), India.

Sample collection: 50 Samples of raw meat of broiler chicken were collected from slaughterhouses and meat shops in the study area by simple random sampling method. Samples were collected in pre-cleaned polythene zip-lock bags, transported

to the laboratory under aseptic conditions and immediately processed for *Proteus* isolation.

Isolation of bacteria: 25 gm of each chicken meat sample was blended and discharged into 225 ml of sterilized buffered peptone water (BPW) and incubated at 37°C for 18 h. After pre-enrichment in BPW, 0.1 ml of pre-enriched sample was transferred to 10 ml of Rappaport-Vassiliadis broth and incubated at 37°C for 24 h. Then the enriched samples were streaked on Hektoen Enteric Agar plates and incubated at 37°C for 24 h. After incubation, typical black colour colonies appeared on the plates selected, and inoculate in nutrient agar and then drew out for further confirmation by PCR analysis.

Molecular Characterization

DNA extraction: The genomic DNA was extracted from bacteria by using ORIGIN Bacterial Genomic DNA isolation Kit as per manufacturer's instruction. Agarose gel electrophoresis was used for the confirmation of the presence of DNA. The amplification reaction was performed by a DNA thermal cycler.

Primer: In the present study, universal 16S rRNA primers for bacteria were used for PCR analysis. The forward primer 27F 5′-AGAGTTTGATCCTGGCTCAG-3′and reverse primer 1492R 5′GGTTACCTTGTTACGACTT-3′ (Weisburg *et al.,* 1991) was used.

DNA Amplification: About 2 ng of genomic DNA was amplified for the 16S rRNA gene using forward and reverse primers. PCR was carried out in a total volume of 50 µl containing 2 ng of genomic DNA (1 µl), 1 µl both forward and reverse primers with at a concentration of 10 µM, 1 µl of dNTPs (2mM), 5 µl of 10X reaction buffer with MgCl2, 0.5 µl Taq polymerase (5 U/µl) and 41.5 µl of water. The cycle conditions followed for amplification in PCR machine: After an initial denaturation at 95°C for 5 min, amplification was made through 30 cycles, each consisting of a denaturation at 95°C for 10 seconds, annealing at 50°C for 1minute, extension at 72°C for 45 seconds and next a final extension at 72°C for 3 minutes.

Agarose Gel Electrophoresis: The amplified DNA products from PCR were resolved on 2% TAE agarose gel stained with ethidium bromide (Sambrook and Russell, 2001) and photographed using a gel documentation system. A Gene Ruler (200bp DNA Ladder) was used to determine the size of the product. As an intercalating agent on DNA, ethidium bromide makes an orange colour to DNA under ultraviolet light. Bands in the gel were visualized by UV transilluminator and photographed with a gel documentation system.

PCR product purification: After confirming the PCR amplification of the corresponding 16S rRNA fragment, the remaining portion of the PCR product was column purified by using GenElute™ PCR Clean-up Kit. The GenElute™ PCR Clea- up Kit is designed for rapid purification of single stranded or double stranded PCR amplification products from other components in the reactions such as excess primers, nucleotides, DNA polymerase, oils, and salts from the PCR products. The purified product was again resolved on 2% agarose gel to ensure the presence of DNA.

DNA sequencing: The purified PCR product was sequenced using the forward and reverse primers used for the PCR using the Sanger's sequencing method (Sanger, 1974) at Sci Genom Labs Private Ltd., Cochin with ABI 3730XL automated sequencer. The trimmed 16S rRNA gene sequences of forward and reverse obtained were aligned using Clustal Omega. Take the aligned region as the final product sequence. The consensus sequence was searched for its similarity using BLAST (Basic Local Alignment Search Tool) programme (Altschul *et al.,* 1990) of NCBI (*http://www.ncbi.nlm.nih.gov*).

RESULTS AND DISCUSSION

Fifty samples were studied and 2 of the isolates were identified as *P. mirabilis* based on the conventional bacteriological analysis and further confirmed by DNA sequence analysis. The molecular level analysis is an excellent way to study the species that contaminate chicken meat. The techniques in molecular biology helped for the identification of bacteria, that the sample (Figure 1) collected from Kottapuram (Malappuram district) was contaminated by *P. mirabilis*. "The sample yielded a product of 801 bp length fragment from the 16S rRNA gene. The BLASTn program states that this species is having 99% sequence similarity to the *P. mirabilis* species reported from Henan, China with Gen Bank accession number is KU942502.1. Figure 2 and 3 are the chromatograms showing forward and reverse sequence of partial 16S rRNA gene of *P. mirabilis* in the sample. *P. mirabilis* is a zoonotic human pathogen of urinary tract infection (UTI), nosocomial or wound infection, therefore, a potential threat to public health.

Figure 1. *P. mirabilis* growth in Hektoen Enteric Agar slant

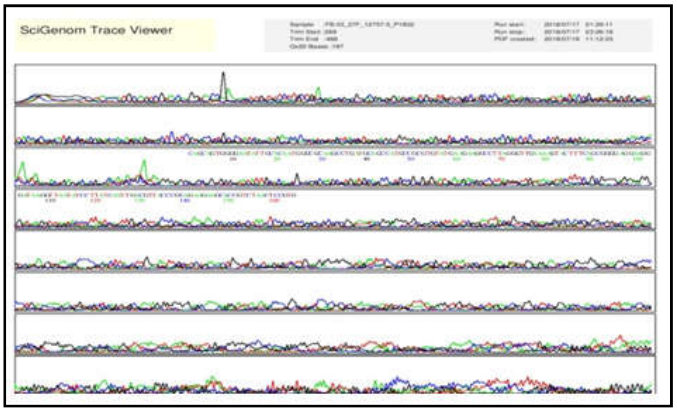

Figure 2. Chromatogram showing forward sequence of partial 16S rRNA of *P. mirabilis* in sample

Several authors reported presence of *P. mirabilis* in poultry meat (Kim *et al.,* 2005; Wong *et al.,* 2013). *P. mirabilis* is also involved in reproductive failure of avian species (Cabassi, 2004).

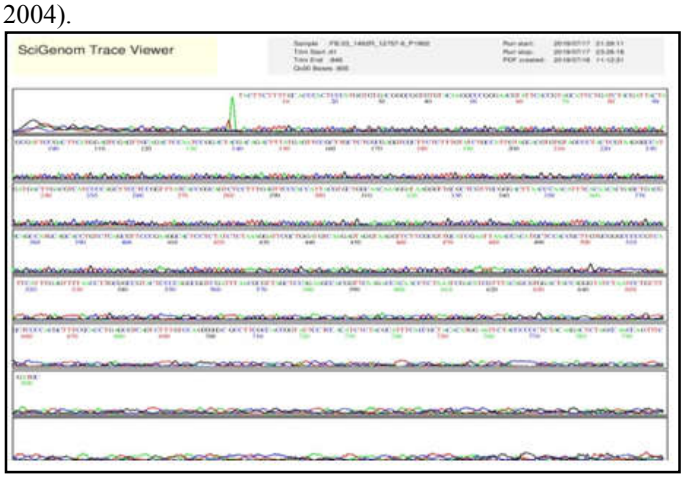

Figure 3. Chromatogram showing reverse sequence of partial 16S rRNA of *P. mirabilis* in sample

In addition, the role of *P. mirabilis* in urinary tract and kidney infection of animals has been previously documented (Gaastra, *et al.,* 1996; Greenberg, *et al.,* 2004; Johnson, *et al.,* 1993; Li, *et al.,* 2002; Martinez, *et al.,* 2003; Nicholson, *et al.,* 1991). *P. mirabilis* is a well known cause of human and animal urinary tract infections and several outbreaks of hospital acquired infections have been attributed to it. However, except for urinary tract infections, the organism is not considered as an important pathogen in veterinary medicine and a recent report of septicaemic *Proteus* infection in Japanese quail (Sah *et al.,* 1983) appears to be the only report of disease due to *P. mirabilis* in an avian species. The high prevalence of *P. mirabilis* isolates in marketed broiler livers, and their similar phenotypic and virulence genes characteristics to that of human isolates, alert us to the need of expanding future research to their adaptability and pathogenesis in different host species (Barbour *et al.,* 2012). *P. mirabilis* also causes huge economic loss to poultry industry as they affect mostly in female fowls and reduce their fertility. This study shows valuable information about the *P. mirabilis* contamination in chicken meat. The level of prevalence can be reduced by adopting hygienic practices during poultry slaughter and it is an important step to decrease *P. mirabilis* related health hazards. Microbial control in every stage of chicken production can control an outbreak of food poisoning and *P. mirabilis* contamination. It is also important from an environmental and occupational health perspective considering the risk involved to those people associated with meat-trade and other related activities.

Conclusion

By examining 50 samples, it was confirmed that 1 sample was contaminated by *P. mirabilis*. This study shows valuable information about the *P. mirabilis* contamination in chicken meat. Maintaining a proper hygienic environment is an important step to avoid *P. mirabilis* related health hazards in humans or spreading to other birds.

Microbial control in each and every stage of chicken production can control an outbreak of food poisoning and reduce the pathogenicity of *P. mirabilis.*

REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. 1990. Basic local alignment search tool. *Journal of Molecular Biology***,** 215 (3): 403-10.

Barbour, E. K., Hajj, Z. G., Hamadeh, S., Shaib, H. A., Farran, M. T., Araj, G., Faroon, O., Barbour K. E., Jirjis, F., Azhar, E., Kumosani, T. and Harakeh, S. 2012. Comparison of phenotypic and virulence genes characteristics in human and chicken isolates of *Proteus mirabilis*. *Pathogens and Global Health,* 106 (6): 352-357.

Cabassi, C. S., Taddei, S., Predari, G., Galvani, G., Ghidini, F., Schiano, E., et al., 2004. Bacteriologic finding in ostrich (Struthio camelus) eggs from farms with reproductive failures. *Avian Dis.,* 48: 716–22.

Gaastra, W., Van Oosterom, R. A., Pieters, E. W, Bergmans, H. E., Van Dijk, L., Agnes, A, et al., 1996. Isolation and characterisation of dog uropathogenic *Proteus mirabilis* strains. *Vet Microbiol.,* 48:57–71.

Greenberg, C. B., Davidson, E. B., Bellmer, D. D., Morton, R. J. and Payton, M. E. 2004. Evaluation of the tensile strengths of four monofilament absorbable suture materials after immersion in canine urine with or without bacteria. *Am J Vet Res.,* 65(6):847–53.

Guentzel, M. N. 1996. Baron S; et al., eds. Escherichia, Klebsiella, Enterobacter, Serratia, Citrobacter, and Proteus. In: *Barron's Medical Microbiology,* (4th ed.). Univ of Texas Medical Branch.

Johnson, D. E., Russell, R. G., Lockatell, C. V., Zulty, J. C., Warren, J. W. and Mobley, H. L. 1993. Contribution of *Proteus mirabilis* urease to persistence, urolithiasis, and acute pyelonephritis in a mouse model of ascending urinary tract infection. *Infect Immun.,* 61(7): 2748–2754.

Li, X., Zhao, H., Virginia Lockatell, C., Drachenberg, C. B, Johnson, D. E. and Mobley, H. L. 2002. Visualization of *Proteus mirabilis* within the matrix of urease-induced bladder stones.

Martinez, I., Mattoon, J. S., Eaton, K. A., Chew, D. J. and Stephen, P. D. 2003. Polypoid cystitis in 17 dogs (1978–2001). *J Vet Intern Med.,* 17:499–509.

Nicholson, E. B., Concaugh, E. A., Mobley, H. L. (1991). *Proteus mirabilis* urease use of a urea-lacZ fusion demonstrates that induction is highly specific for urea. *Infect Immun.,* 59:3360–3365.

Sah, R. L., Mall, M. P. and Mohanty, G. C. 1983. Septicemic Proteus infection in Japanese quail chicks (Coturnix coturnix japonica). *Avian Diseases.,* 27: 296 – 300.

Sambrook, J. and Russell, D. W. 2001. Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press, New York.

Sanger, F. 1974. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *Journal of Molecular Biology***,** 94 (3): 441-448.

Weisburg, W. G., Barns, S. M., Pelletier, D. A. and Lane, D. J. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.,* 173: 697–703. (doi:10.1128/jb.173.2. 697-703.1991).

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