Acta Scientific Pharmaceutical Sciences

Volume 1 Issue 5 November 2017

Research Article

Molecular Characterization and Antibiotic Resistance of Bacteria Isolated from Food Samples from Hooghly District of West Bengal State, India

Raktima Bandyopadhyay[1], Syed Afrin Azmi[2], Soumendranath Chatterjee[2] and Shyamapada Mandal[1]*

[1]*Department of Zoology, University of Gour Banga, Malda, India*

[2]*Department of Zoology, University of Burdwan, Burdwan, India*

***Corresponding Author:** Shyamapada Mandal, Laboratory of Microbiology and Experimental Medicine, Department of Zoology, University of Gour Banga, West Bengal, Malda, India. E-mail: samtropmed@gmail.com

Received: August 15, 2017; **Published:** October 18, 2017

Abstract

The food contamination with pathogenic bacteria and their antibiotic resistance have been reported worldwide. The present study dealt with the isolation and characterization of the bacterial contaminants of food collected from Hooghly district of the West Bengal, India. Two bacterial isolates (GUTKR5 and GUTKR7) were procured from the food samples (vegetables and soups) screened. Neighbor joining tree analysis revealed that GUTKR5 isolate branched with *Pseudomonas aeruginosa* JN996498 strain with 87% bootstrap value. From biochemical and molecular characterization, the bacterial isolates GUTKR5 and GUTKR7 were identified as *Pseudomonas aeruginosa* and *Aeromonas veronii*, respectively. The disc diffusion susceptibility test revealed that both the bacterial isolates, *Pseudomonas aeruginosa* GUTKR5 and *Aeromonas veronii* GUTKR7, showed resistance to AM and PC, while *Pseudomonas aeruginosa* GUTKR5 and *Aeromonas veronii* GUTKR7 had further resistance to CP and VM, respectively, too; all the bacterial isolates were sensitive to CM, TC, EM and GM. Thus, regular surveillance for drug resistance among potential pathogenic bacteria isolated from different food samples might help combat food borne infection caused by the MDR bacterial pathogens.

Keywords: Food Borne Bacteria; Multidrug Resistant; *Pseudomonas aeruginosa; Aeromonas veronii;* MAR Index; 16S rRNA Gene Identification

Abbreviations

AM: Ampicillin; CLSI: Clinical and Laboratory Standards Institute; CM: Chloramphenicol; CP: Ciprofloxacin; EM: Erythromycin; GM: Gentamycin; MAR: Multiple Antibiotic Resistance; PC: Penicillin G; PCR: Polymerase Chain Reaction; RFLP: Restriction Fragment Length Polymorphisms; TC: Tetracycline; VM: Vancomycin; ZDI: Zone Diameter of Inhibition

Introduction

Food-borne illness due to bacterial infection has been the major public health concern worldwide [1]; approximately 76 million cases of food-borne disease occur every year, and as many as 3.25 × 10^5 hospitalization and 5.2 × 10^5 death [2]. Health economists have acknowledged that it is very difficult to estimate the full cost of food-borne diseases [3], and estimates of the financial cost vary across publications. The great concern of episodes of food borne illnesses has been reported from USA and Australia [4, 5]. A major proportion of the global incidences of food borne diseases attributed to the contamination of food and drinking water [6]. Bacteria, such as *Salmonella* sp., *Escherichia coli* and *Staphylococcus aureus* have been reported to cause food poisoning and food-borne diseases [7]. Moreover, increased incidence of food borne diseases cause the increased emergence of new food borne pathogens, such as *Yersinia enterocolitica, Listeria monocytogenes, Staphylococcus enteritidis* and *Campylobacter jejuni* [8-10]. The bacterial contamination of various kinds of foods has been reported by the earlier authors from different parts of India. Tambekar *et al.* [11] revealed the presence of both gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli, Klebsiella* sp. and *Pseudomonas* sp.) bacteria in 'panipuri' water. The predominant bacteria isolated from meat samples were *Escherichia coli, Salmonella* spp., *Staphylococcus aureus* and *Pseudomonas* sp., as per the report of Thanigaivel *et al.* [12]. Hemalata *et al.* [13] reported the presence of multidrug resistant bacteria including *Pseudomonas* spp. from various food samples. It has been reported that some of the motile *Aeromonas* spp. are regarded as emerging food pathogens [14], and most of the human gastrointestinal illnesses are linked to the infection with *Aeromonas hydrophila, Aeromonas caviae* and *Aeromonas veronii* [15].

However, no report has been made, from our part of the globe, on the bacterial contaminants of foods: vegetables or soups, and thus, the antibiotic resistance of food borne bacteria. Therefore, the current study has been undertaken to explore the antibiotic resistance of bacteria isolated from some ready-to-eat food (rice, bread, vegetable and soup) samples collected from Tarakeswar locality of Hooghly district, West Bengal, India.

Materials and Methods

Collection of food samples

The food samples (n = 12) from the items: rice, bread, vegetable and soup (fresh as well as unpreserved kept for later usage), prepared for the consumption of children, were collected from do-

mestic levels, by active surveillance, at Loknath area of Tarakeswar block, Hooghly district of West Bengal state, India, and were subjected for bacteriological processing.

Isolation and identification of bacteria

The food samples were inoculated into nutrient broth (Hi-Media, India) and incubated at 35 °C for 24h, for enrichment. The pure bacteria cultures were obtained by streak-plate dilution method as described in our previous publication [16], and the bacterial isolates, which were designated as GUTKR5 and GUTKR7, were stored in cystine tryptone agar (Hi-Media, India) stabs. The isolated bacteria were identified following gram-staining, biochemical tests and sugar fermentation [17,18].

Molecular identity of the isolated bacteria

The molecular identity of the bacterial isolates (GUTKR7 and GUTKR5) was determined by 16s rRNA gene identification. The genomic DNA from GUTKR7 and GUTKR5 food bacterial isolates was extracted following standard methodology [19], PCR amplification of the 16S rRNA gene was done and sequence was generated utilizing universal primer. The phylogenetic tree was constructed following Saitou and Nei [20], and Tamura *et al.* [21].

Antibiotic Susceptibility

The antibiotic susceptibility for the food bacterial isolates, GUTKR5 and GUTKR7, was determined by disc diffusion technique [22], using Mueller-Hinton agar (Hi-Media, India) plates, against AM, CM, CP, EM, GM, PC, TC and VM, as described earlier [16]. The ZDI values obtained around the test antibiotic discs for the isolated bacteria were interpreted according to the CLSI criteria [23], in order to categorize the bacterial isolates as resistant, sensitive or intermediately susceptible.

Determination of multiple antibiotic resistance indices

The MAR indices for the two isolated bacteria (GUTKR5 and GUTKR7) were calculated as per the formula stated elsewhere [24,25], and interpreted according to Krumperman [25]: MAR index ≤ 0.2 was considered low risk, and ≥ 0.2 was indicated high risk of contamination.

Results and Discussion

Two of the bacterial isolates, GUTKR5 and GUTKR7, were found to be the most prevalent in the collected food samples (vegetables and soups); no bacterial contaminants were found in rice and bread. Both of the isolated bacteria were gram-negative non-spore forming rod-shaped showing positivity to catalase, oxidase, citrate, nitrate, and gelatin hydrolysis tests, and negativity to MR, H_2S production and urease tests; GUTKR7 isolate was indole positive. Both of the isolates showed β-hemolytic activity on sheep-blood agar plate (available from Hi-Media, India), while neither was lactose fermenting.

Figueras *et al.* [26] reported the fast and low-cost technique on the basis of RFLP of the 16S rDNA amplified PCR. In the instant study, the neighbor joining tree analysis revealed that GUTKR5 isolate branched with *Pseudomonas aeruginosa* JN996498 strain, having 87% bootstrap value, and the cluster containing GUTKR5 isolate and *Pseudomonas aeruginosa* JN996498 strain branched with the cluster containing other *Pseudomonas* spp. sequences obtained from the Genbank (Figure 1). The food bacterial isolate GUTKR7 branched with *Aeromonas veronii* GQ334329 strain, having 64% bootstrap value, and the cluster containing GUTKR7 isolate and *Aeromonas veronii* GQ334329 strain branched with *Aeromonas veronii* KT371350 strain (Figure 2). The restriction enzyme maps for the GUTKR5 and GUTKR7 isolates, represented in Figure 3 and Figure 4 respectively, exhibited cutting sites of different restriction enzymes within the sequences.

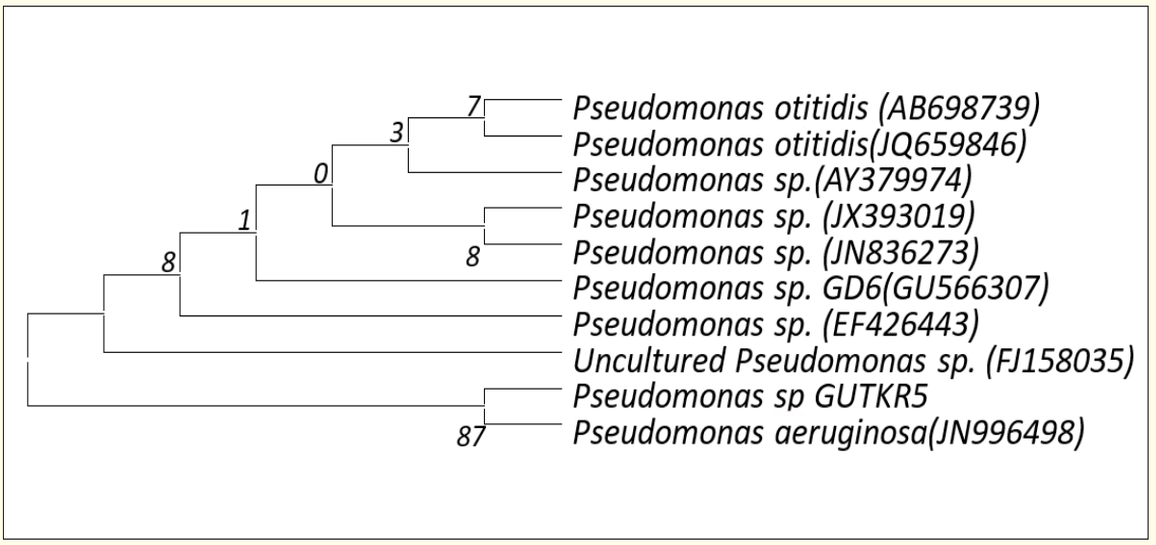

Figure 1: Neighbor joining tree for GUTKR5 isolate constructed through 16S rRNA gene sequencing.

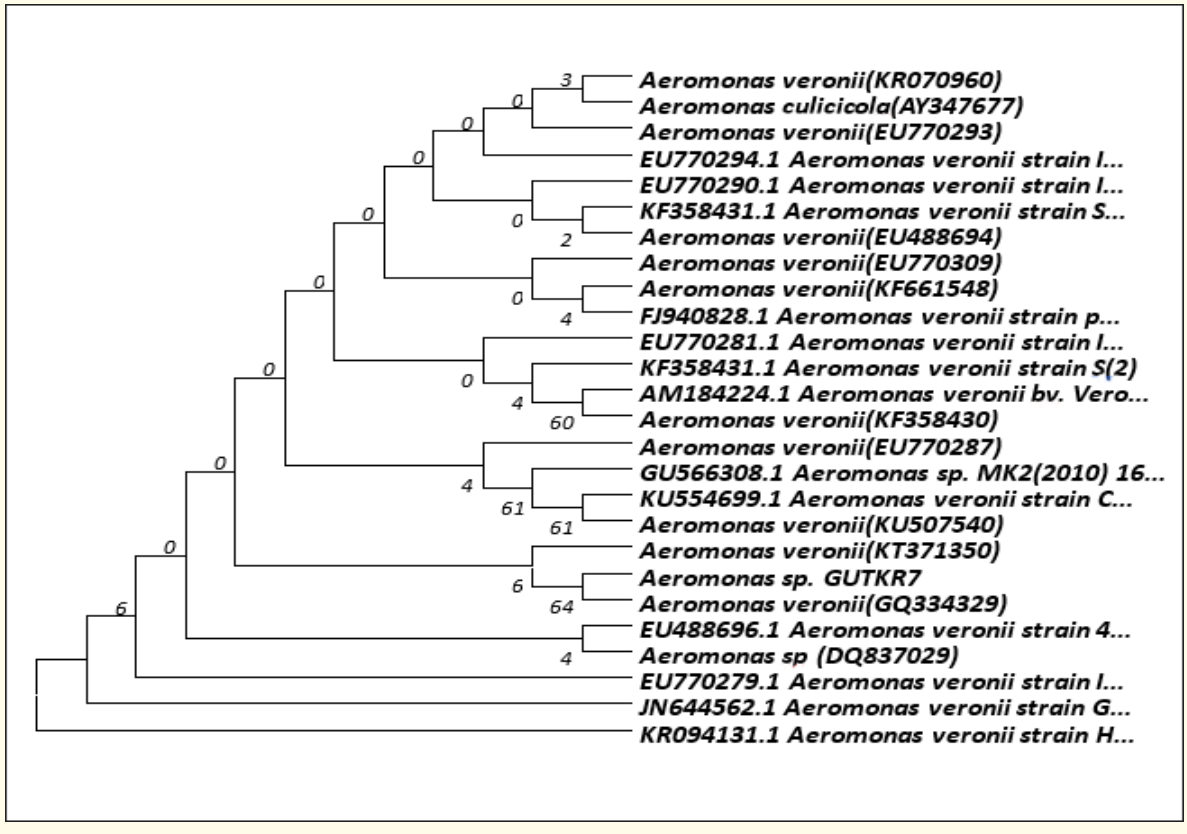

Figure 2: Neighbor joining tree for GUTKR7 isolate constructed through 16S rRNA gene sequencing.

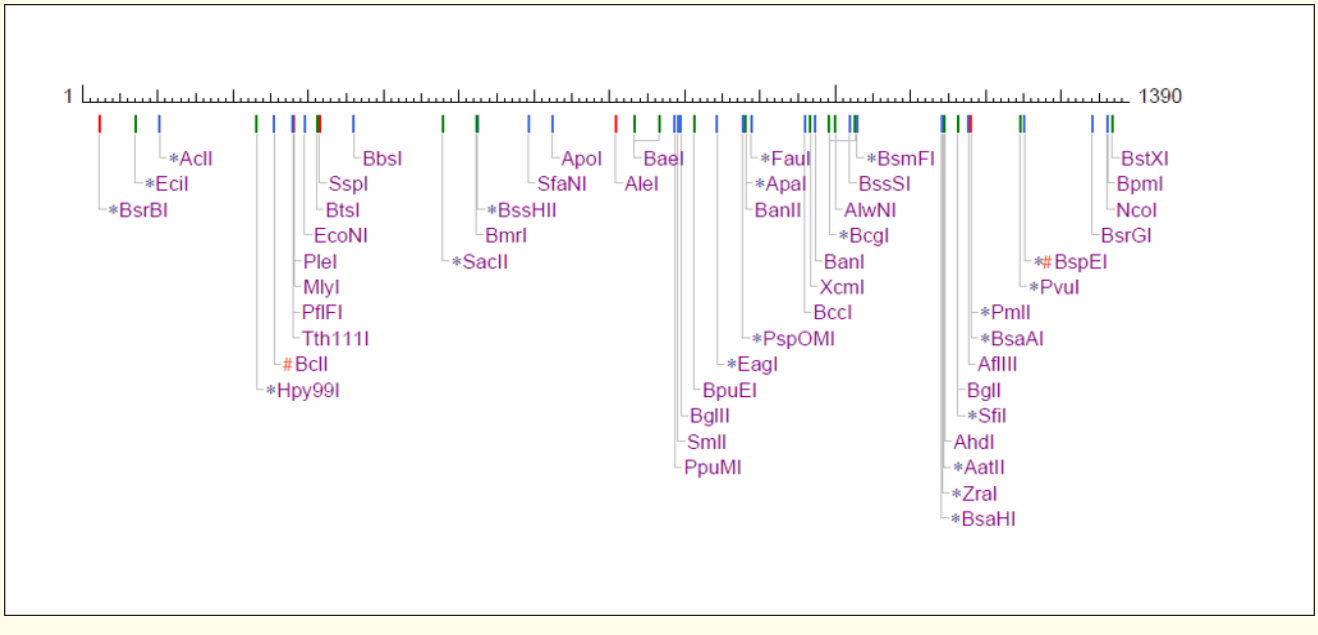

Figure 3: Restriction enzyme map of the 16S rRNA gene sequence of GUTKR5 isolate.

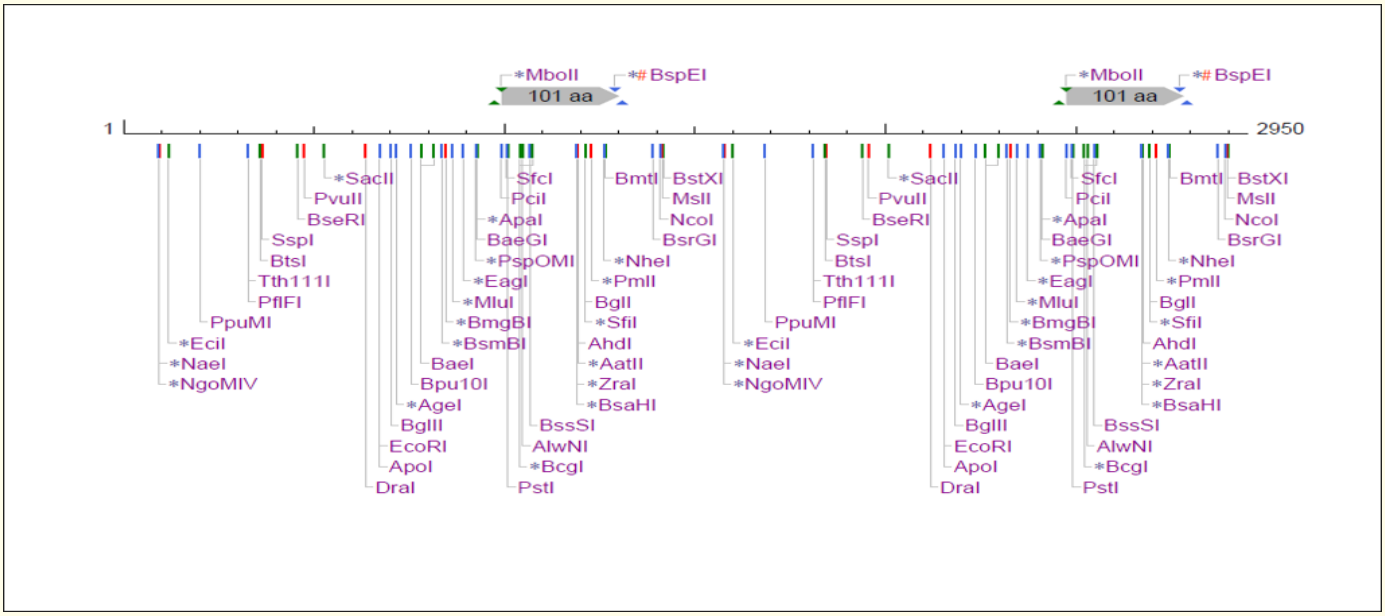

Figure 4: Restriction enzyme map of the 16S rRNA gene sequence of GUTKR7.

In the current study, based upon the findings from the conventional identity tests (gram-staining, cultural and biochemical), and the phylogenetic analysis, the GUTKR5 and GUTKR7 isolates were identified as *Pseudomonas aeroginosa* and *Aeromonas veronii*, respectively. It has been reported that in 1960s, *P. aeruginosa* emerged as a significant pathogen to cause human infections, and currently, the bacterium has well been reported to cause nosocomial and community infections, as well as food-borne infection, having the capacity to form biofilm and possessing an array of pathogenic determinants [27-29]. The species of the genus *Aeromonas* are omnipresent, water-borne bacteria, found in foods (fish, seafood, meat, vegetables and processed foods), and are potential to represent serious problem in food, because of their capacity to produce exotoxins. The *Aeromonas* spp. are emerging as important human pathogens, causing several extra-intestinal and systemic infections (septicemia, hemolytic uremic syndrome and eye infections) and gastrointestinal infections (self-limiting liquid diarrhea to a more severe and invasive diarrhea) as well [30]. Wang *et al*. [31] reported β-hemolytic activity in 6 *Aeromonas caviae* isolates, among a total of 35, from clinical origin. Stratev *et al.* [15] reported that the gastrointestinal illnesses in humans are associated with the infection of different species of the genus *Aeromonas*, such as *A. hydrophila, A. caviae* and *A. veronii*. In the current study, the β-hemolytic and gelatin hydrolysis properties of the isolated bacteria, *P. aeruginosa* and *A. veronii*, from ready-to-eat food samples indicated their pathogenic nature, and therefore, their existence in ready-to-eat food samples might pose serious threats to humans.

Sarwat *et al.* [29] reported that the nosocomial *P. aeruginosa* isolates had more resistance to antibiotics (ceftazidime, tobramycin, ticarcillin, ticarcillin/clavulanic acid piperacillin/tazobactam) compared to the community isolates. Hemalata *et al.* [13] isolated *Pseudomonas* spp., from various food samples, showing multidrug resistance to cefotaxime, co-trimoxazole, meropenem, imipenem, gentamicin and tetracycline. In this communication, both the food bacterial isolates, *Pseudomonas aeruginosa* GUTKR5 and *Aeromonas veronii* GUTKR7, showed resistance to AM and PC, while *Pseudomonas aeruginosa* GUTKR5 and *Aeromonas aeruginosa* GUTKR7 had further resistance to CP and VM, respectively, too; all the bacterial isolates were sensitive to CM, TC, EM and GM. Krumperman [25] originally demonstrated the idea of bacterial MAR indices among *E. coli* isolates. In our previous publication, the MAR indices for eye-cosmetic bacteria have been reported, with the highest value (≥ 0.4) recorded in case of *P. aeruginosa* [16]. Tripathi *et al.* [32] reported the MAR indices of ≥ 0.3 among most of the clinical isolates of *P. aeruginosa.* No report has been available from this part of the globe on MAR indices of food borne bacteria. As

has been reported by Vivekanandhan *et al.* [33], the MAR indices for most of the isolated *Aeromonas hydrophila* strains had been recorded as ≥ 0.33, and thus suggesting the origin of the bacteria from high-risk source of contamination. In this study, the *Pseudomonas aeruginosa* GUTKR5 and *Aeromonas veronii* GUTKR7 isolates were MDR, for which the MAR index was recorded as 0.375, thereby demonstrating their (food borne bacteria) plausible origin from highly antibiotic-contaminated sources. Thus, in order to prevent the bacterial contamination of foods and their infection to humans, application of biotherapeutic agents has been commended [34-36].

Conclusion

The occurrence of food contamination with potential MDR pathogenic bacteria, *Pseudomonas aeruginosa* and *Aeromonas veronii,* reflects the unhygienic and poor sanitary practices of the food handlers during preparation, packing or serving. Thus, this study sincerely recommends the maintenance of hygienic setting during preparation, packing or serving of food by the people, in the community, in order to fight the prevalence of MDR food bacteria having the capacity to cause life-threatening infections. However, for proper awareness development among the people and to combat the bacterial food borne illnesses, extensive surveillance of antibiotic resistance in bacterial food contaminants, in different settings, is mandatory.

Author Contributions

Raktima Bandyopadhyay performed experimental works and co-wrote the paper; Syed Afrin Azmi performed experimental works; Soumendranath Chatterjee characterized and analyzed the molecular identity of test bacteria; Shyamapada Mandal designed the study, wrote and discussed the entire paper.

Conflicts of Interest

There was no conflict of interest.

Bibliography

1. Altekruse SF., *et al.* "The changing epidemiology of foodborne diseases". *The American Journal of the Medical Sciences* 311.1 (1996): 23-29.

2. Frank LB., *et al.* "Surveillance of food bourne disease III". *Journal of Food Protection* 60.6 (1997): 701-714.

3. Sockett PN., *et al.* "The social and economic impact of salmonellosis". *Epidemiology and Infection* 107 (1991): 335-347.

4. Sockett PN. "Food poisoning associated with manufactured foods in England and Wales, 1980–1989". *Communicable Disease Report* 1 (1991): 105-R109.

5. Australia New Zealand Food Authority (ANZFA). "Proposal to develop a national food hygiene standard" (1996): 1-44.

6. World Health Organisation. "Foodborne Disease: A focus for health education" (2000).

7. Foskett P., *et al.* "The theory of catering" 10th ed. Hodder and Stoughton: London (2003): 531.

8. Bryan FL. "Impact of food borne diseases and methods of evaluating control program". *Journal of Environmental Health* 40 (1980): 315-323.

9. Nelson JD. "Etiology and epidemiology of diarrhoeal disease in the United States". *American Journal of Medicine* 78.6 (1985): 76-80.

10. Crerar SK., *et al.* "Foodborne disease: current trends and future surveillance needs in Australia". *Medical Journal of Australia* 165 (1996): 672-675.

11. Tambekar DH., *et al.* "Bacteriological quality of street vended food panipuri: a case study of Amravati city (MS) India". *Bioscience Discovery* 2.3 (2011): 350-354.

12. Thanigaivel G., *et al.* "Isolation and characterization of microorganisms from raw meat obtained from different market places in and around Chennai". *Journal of Pharmaceutical, Chemical and Biological Sciences* 3.2 (2015): 295-301.

13. Hemalata VB., *et al.* "Isolation and identification of food borne pathogens from spoiled food samples". *International Journal of Current Microbiology and Applied Sciences* 5.6 (2016): 1017-1025.

14. Gheghesh KS., *et al.* "Aeromonas infections in developing countries". *Journal of Infection in Developing Countries* 2.2 (2008): 81-98.

15. Stratev D., *et al.* "Prevalence and survival of Aeromonas spp. in foods - a review". *Revue de Médecine Vétérinaire* 163.10 (2012): 486-494.

16. Nandi S., *et al.* "Bacteriological profiling of commercially available eye cosmetics and their antibiotic susceptibility pattern". *Translational Biomedicine* 7 (2016): 3.

17. Holt JG. "Bergey's Manual of Systematic Bacteriology". Williams and Wilkins, Baltimore (1984).

18. Forbes BA., *et al.* "Bailey and Scott's Diagnostic Microbiology". 12th Edition, Mosby (Elsevier), USA (2007).

19. Janssen K. "Current protocols in molecular biology" Greene Publ. Assoc. Inc. and John Wiley Sons Inc, New York (1994).

20. Saitou N., *et al.* "The neighbor-joining method: a new method for reconstructing phylogenetic trees". *Journal of Molecular Biology* 4.4 (1987): 406-425.

21. Tamura K., *et al.* "MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0". *Molecular Biology and Evolution* 24.8 (2007): 1596-1599.

22. Bauer AW., *et al.* "Antibiotic susceptibility testing by a standard single diffusion method". *American Journal of Clinical Pathology* 45.4 (1966): 494-496.

23. Clinical and Laboratory Standards Institute (CLSI): Performance standards for antimicrobial susceptibility testing; 21st informational supplement M100S21. CLSI; Wayne, Pa (2011).

24. Maloo A., *et al.* "Occurrence and distribution of multiple antibiotic-resistant bacteria of Enterobacteriaceae family in waters of Veraval coast, India". *Environmental and Experimental Biology* 12 (2014): 43-50.

25. Krumperman PH. "Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of fecal contamination of foods". *Applied and Environmental Microbiology* 46.1 (1983): 165-170.

26. Figueras MJ., *et al.* "Use of restriction fragment length polymorphism of the PCR-amplified 16S rRNA gene for the identification of Aeromonas spp". *Journal of Clinical Microbiology* 38.5 (2000): 2023-2025.

27. Alhazmi A. "Pseudomonas aeruginosa – Pathogenesis and Pathogenic Mechanisms". *International Journal of Biology* 7.2 (2015): 44-67.

28. Gellatly SL., *et al.* "Pseudomonas aeruginosa: new insights into pathogenesis and host defences". *Pathogens and Disease* 67.3 (2013): 159-173.

29. Sarwat T., *et al.* "A Comparative Study of Antibiogram of Pseudomonas aeruginosa in hospital and community acquired infections". *International Journal of Current Microbiology and Applied Sciences* (2015) Special Issue-1: 286-291.

30. Tomas JM. "The main Aeromonas pathogenic factors". *ISRN Microbiology* (2012).

31. Wang G., *et al.* "Characterizationof cytotoxic, hemolytic Aeromonas caviae clinical isolates and their identification by determining presence of a unique hemolysin gene". *Journal of Clinical Microbiology* 34.12 (1996): 3203-3205.

32. Tripathi P., *et al.* "Antibiotic resistance pattern of Pseudomonas aeruginosa isolated from patients of lower respiratory tract infection". *African Journal of Microbiology Research* 5.19 (2011): 2955-2959.

33. Vivekanandhan G., *et al.* "Antibiotic resistance of Aeromonas hydrophila isolated from marketed fish and prawn of South India". *International Journal of Food Microbiology* 76 (2002): 165-168.

34. Sircar B., *et al.* "Screening of Elaeocarpus floribundus fruit extracts for bioactive phytocomponents and antibacterial activity against food-borne bacteria". *International Journal of Research in Medical Sciences* 5.8 (2017): 3665-3671.

35. Sircar B., *et al.* "Biosynthesis of Elaeocarpus floribundus mediated silver nanoparticles with broad antibacterial spectrum. *International Journal of Pharma and Drug Development* 1.2 (2017): 59-67.

36. Mandal S., *et al.* "Can honey combat life threatening bacterial infections to humans". *International Journal of Pharma and Drug Development* 1.3 (2017): 6-9.

Volume 1 Issue 5 November 2017