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Review Article

# Cultural growth and isolation of food borne bacteria

**Ghalia Nawal**

Department of Zoology, Lahore College for Women
University LCWU, Lahore, Pakistan.
*Corresponding author: *ghalianawal@yahoo.com*

## Abstract

Food harbors a variety of bacteria some of them are common commensal while others are pathogenic and many outbreaks of food borne illness are reported annually which are related directly to the consumption of a specific food item which has now become a major concern globally. Through proper investigation with advancement in Biotechnology has revealed the presence of pathogenic food borne bacteria like *Compylobacter jejuni*, *Listeria monocytogenes* and *Staphylococcus aureus* in many food items. Isolation requires cultural growth of these bacteria first on agar plates then on selective and differential media according to specific conditions require by each bacterium to grow. Further confirmation can be achieved with help of Biochemical and serological confirmatory tests.

**Keywords:** *Compylobacter jejuni*, *Listeria monocytogenes* and *Staphylococcus aureus.*

## Introduction

### *Campylobacter jejuni*

*C.jeujni* is one of the commonest causes of gastroenteritis throughout the world. It is a Gram-negative; non spore forming, helical, curved, fastidious and micro-aerophilic pathogen of mainly animal origin associated with poultry meat. It has been gaining recognition and importance as an emerging food borne illness pathogen for causing over three times more food poisoning cases annually than *Salmonella. It grows* within a temperature range between 37° and 42°C.After taking inoculums from incubated test samples and streaking them on agar plates will result in their initial growth afterwards various types of selective and differential media can be used to confirm their presence. Enrichment is recommended to enhance the culture sensitivity of potentially environmentally stressed organisms. For recovery modified charcoal, cefoperazone , desoxycholate agar (mCCDA), is the most recommended medium, although alternative selective media may be used are classified into two main groups:

- blood-containing media
- Charcoal containing media.

Blood components and charcoal serve to provide micro-aerophilic conditions by removing toxic oxygen derivatives. Most media are commercially available. The selectivity of the media is achieved by the use of antibiotics e.g Cefalosporins (generally cefoperazone).sometimes in combination with other antibiotics (e.g. vancomycin, trimethoprim). Cycloheximide (actidione) and more recently amphotericin B are used to inhibit yeasts and molds. Examples of selective blood-containing solid media are Preston agar, Skirrow agar, Butzler agar and Campy-cefex .While examples of charcoal-based solid media is mCCDA (modified charcoal cefoperazone deoxycholate agar) , Karmali agar or CSM (charcoal-selective medium) and CAT agar (cefoperazone, amphotericin and teicoplanin) facilitating growth of *Campylobacter* species.

Another method developed by Steele and McDermott is Passive filtration which obviates the need for selective media and is very useful for the isolation of antimicrobial-sensitive Campylobacter species. This method is comparatively cheaper as it does not involve

expensive selective media so is more convenient to be used in laboratories with fewer resources. For passive filtration, feces are mixed with PBS (phosphate buffered saline solution) approximately 1/10 dilution to produce a suspension. Then 100 µl of this suspension is carefully layered on to a 0.45 or 0.65 µm filter, which has been previously placed on top of a non-selective blood agar plate. Care must be taken not to allow the inoculums to spill over the edge of the filter. The bacteria are allowed to migrate through the filter for 30–45 minutes at 37°C or room temperature. The filter is then removed, the fluid that has passed through the filter is spread with a sterile glass or plastic spreader, and the plate is incubated micro-aerobically at 42°C (Chaban, Guerra, Hendrick, Waldner & Hill, 2013).

For incubation at micro-aerobic atmospheres of 5–10% oxygen, 5–10% carbon dioxide is required for optimal growth. Repeated gas jar evacuations followed by atmosphere replacement with bottled gasses are used. For confirmation a pure culture is required for confirmatory but initially it can be confirmation can be obtained by direct Microscopic examination of bacterial colonies.On blood-containing agars e.g. skirrow agar characteristic Campylobacter colonies are slightly pink, round, convex, smooth and shiny, with a regular edge. On charcoal-based media such as mCCDA, colonies are grayish, flat and moistened, with a tendency to spread, and may have a metal sheen. For motility confirmation material from a suspect colony is suspended in saline and evaluated with help of preferably by a phase-contrast microscope revealing the presence of spiral/curved slender rods with a corkscrew-like motility. Older cultures show less motile forms (Gblossi Bernadette et al., 2012)
Biochemical confirmatory tests involve oxidase test performed with oxidase reagent on filter paper color change to violet or deep blue color within 10 seconds is a positive reaction. Latex agglutination tests are also commercially available for confirmation.

## *Listeria monocytogenes*

*Listeria monocytogenes* is an unpopular cause of illness in population. It is an infectious agent of listeriosis, showing rod shaped structure and appears gram-positive upon staining. It can survive normal refrigeration. It can causing severe disease in individuals with weakened immunity systems like newborns and women. They are intracellular pathogens that use actin filaments within the host cell for their motility. *Listeria* are non spore-forming and non-branching microaerophilic, facultative anaerobes that occur individually or form short chain.The organism has a multi factorial virulence system, with the thiol-activated hemolysin and listeriolysin O, playing a crucial role in the organism's ability to multiply within host phagocytic cells and to spread into other cells. The pathogen can be isolated from a very wide range of foods specially refrigerated ones including milk, soft cheeses, ice cream and other seafood. *L. monocytogenes* is usually found only in low numbers (less than 10/g) in general foods while in milk and soft cheese contain levels of 10,000/g.

The growth and survival of *L. monocytogenes* is influenced by a number of factors including temperature, pH, water activity, salt and the presence of preservatives. The temperature range for growth is between -1.5 and 45°C, with the optimal temperature of 30–37°C. High temperature e.g. 50°C are lethal to *L. monocytogenes*. Freezing can also lead to a reduction in *L. monocytogenes* numbers.They will grow in a broad pH range of 4.0–9.6 with relative tolerance to acidic conditions at higher temperatures, having ability to grow at water activity of 0.90(Lado and Yousef 2007).*L. monocytogenes* is reasonably tolerant to salt and can grow in 13–14% sodium chloride (Farber et al. 1992). *L. monocytogenes* can grow under both aerobic and anaerobic conditions, with better growth in an anaerobic environment (Sutherland et al. 2003; Lado and Yousef 2007). There are at least 13 different serotypes of *L. monocytogenes* as 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e and 7. While serotypes most often associated with human illness are 1/2a, 1/2b and 4b as reported by FDA in 2012.

Conventional method of their growth is to culture it on plating media..The selective media prepared by McBride and Girard was among the first solid media used for recovering them. Another early medium contained nalidixic acid, polymyxin B and Arciflavin.A modification of MacBride's Agar (MMA) was developed which contain phenylethanol,glycine anhydride,lithium chloride and cycloheximide as selective agents. After incubation on MMA-plates listeria species They *Listeria* colonies are small, smooth, granular and bluish-gray when the plates are examined by oblique 45 degree transmit light with help of a stereo-microscope.While for optimal isolation of *Listeria* species, direct plating of samples(more preferably faeces in suspecting of food borne illness) onto blood agar is recommended. Selective media such as Oxford agar or PALCAM are recommended, as well as enrichment in a *Listeria* selective broth. Some specific media for *Listeria* are

fraser broth base, *Listeria* agar base broth oxford, *Listeria* Agar base palcam and Tryptone soy yeast extract agar (Curtis, 1996).

### *Staphylococcus aureus*

*S.aureus* is responsible for massive outbreaks of food poisoning by the production of enterotoxins mostly through enterotoxin A and H. This bacterium is gram positive, coccus, facultative anaerobe and non motile .Human beings are its natural carrier about 30 to 50 % of population harbors them. It is commonly found on skin, nostrils, hairs and hair of warm blooded animals; hence it can contaminate the food easily during processing and preparation of food resulting in food borne illness. The percentage of Enterotoxigenic strains in food samples is about 25 % (Bergdoll, 1989). Spectrum of illness caused by *Staphylococcus* species ranges from common infections to staphylococcal food poisoning and toxic shock syndrome (Argudín, Mendoza & Rodicio, 2010)
This bacterium is able to grow at a variety of temperature ranges i.e. from 7$^{o}$ to 48.5$^{o}$C and optimally at 37$^{o}$C.Since these bacteria belong to non-spore forming specie their growth can easily be avoided with heat or high temperature treatment, acting as a physical agent of control (Schmitt et al., 1990).They are able to grow at the PH range of 4.2 to 9 but likewise optimum temperature similar to temperature of human beings they grow optimally at PH 7 to 7.5.

In 1992 Hill and Whito suggested the use of media with higher salt concentration as much as 15% for inhibiting growth of gram negative bacilli and promoting growth of gram positive coccid bacteria for their recover and isolation. They studied gram positive bacteria can tolerate higher salt concentrations and they are viable up to 20% sodium chloride base agar medium with mild bacteriostatic action. Later Koch in 1942 prescribed the use of Nutrient agar medium with concentration of 7.5% sodium chloride for selective isolation of *Staphylococcus aureus* (Maitland & Martyn, 1948).Since then Up till now 7.5 % salt containing (sodium chloride) agar base medium is use for cultural growth and selective isolation of this bacterium. The most widely used Agar base medium for this purpose is Mannitol Salt Agar containing Mannitol sugar and 7.5 % salt (NaCl) concentration at which other bacteria rather than *Staphylococcus spp.* cannot tolerate highly saline and hypertonic environment because of the absence of slime layer .Another advantage of using this medium is that it can differentiate between species of *Staphylococcus* on the basis of fermentation of Mannitol sugar resulting in formation of Alcohal. *Staphylococcus aureus* can ferment mannitol sugar and does not change the color of the medium with production of yellow colonies while non pathogenic form i.e. *Staphylococcus epidermidis* cannot ferment mannitol sugar and produce pink colonies.

Biochemical isolation methods for *S.aureus* species involve Catalase test, Coagulation test, pigmentation production and determination of blood cell Hemolysis on Blood sheep Agar plates

## References


Argudín, M.,Mendoza, M., & Rodicio, M. (2010).Food Poisoning and Staphylococcus aureus Enterotoxins. *Toxins*, *2*(7), 1751-1773.

Bergdoll, M.S. (1983). Enterotoxins. In: Staphylococci and Staphylococcal Infections (Easman, C.S.F. and Adlam, C., eds.). Academic Press, London, UK, pp. 559-598.

Chaban, B., Guerra, A., Hendrick, S., Waldner, C., & Hill, J. (2013). Isolation rates of Campylobacter fetus subsp venerealis from bovine preputial samples via passive filtration on nonselective medium versus selective medium, with and without transport medium. *American Journal Of Veterinary Research*, *74*(8), 1066-1069.

Curtis, G. (1996). Int. J. Food Microbiology Erratum to “culture media and methods for the isolation of Listeria monocytogenes” 26 (1995) 1–13. *International Journal Of Food Microbiology*, *28*(3), 433.

Farber (1992). Minimum water activity requirements for the growth of Listeria monocytogenes. *Letters In Applied Microbiology*, *15*(3), 103-105. doi:10.1111/j.1472-765x.1992.tb00737.x

Gblossi Bernadette, G., Eric Essoh, A., Elise Solange, K., Natalie, G., Souleymane, B., Lamine Sébastien, N., & Mireille, D. (2012). Prevalence and Antimicrobial Resistance of Thermophilic Campylobacter Isolated from Chicken in Côte d’Ivoire. *International Journal Of Microbiology*, *2012*, 1-5.

Lado, B., & Yousef, A. (2007). Selection and Identification of a Listeria monocytogenes Target Strain for Pulsed Electric Field Process Optimization. *Applied And Environmental Microbiology*, *69*(4), 2223-2229.

Maitland, H., & Martyn, G. (1948). A selective medium for isolating staphylococcus based on the differential inhibiting effect of increased

concentrations of sodium chloride. *J. Pathol.*, *60*(4), 553-561.

Schmitt, M., Schuler-Schmid, U. and Scmidt-Lorenz, W. (1990). Temperature limits of growth, TNase, and enterotoxin production of Staphylococcus aureus strains isolated from foods. Int. J. Food Microbiol. 11: 1-19.

Sutherland PS, Miles DW, Laboyrie DA (2003) Listeria monocytogenes. Ch 13 In: Hocking, AD (ed) Foodborne microorganisms of public health significance. Australian Institute of Food Science and Technology (NSW Branch), Sydney, p. 381–443

http://www.funpecrp.com.br/gmr/year2003/vol1-2/sim0009_full_text.htm