farmers in agriculture gave tangible benefits in terms of increase in static water levels in many bore wells in the village and revived many dry wells. Figure 3 presents the water level trends in OBWs in the village from February 2012 to December 2016 (ref. 8). To convince other farmers about the efficacy of this technology, yield demonstrations were organized for groups of farmers in farm lands where bore well recharge structures were built. After participating in one such demonstration, the National Bank for Agriculture and Rural Development (NABARD), with technical support from SuGWM project, replicated the technology in the Karimnagar district of Telangana state in 2016.

The bore well recharge technology was found effective in hard-rock hydrogeological conditions, especially in low-rainfall regions with intense rainfall events. By recharging a few identified bore wells, groundwater condition could be revived to enhance agricultural productivity and farmers' income. Contamination of deep groundwater due to the use of untreated surface run-off is a potential concern in this technique. Thus, it is highly desirable to avoid the technique in areas where higher levels of biological and chemical contaminants are noticed in run-off water. In contrast to known methods, the technique involves re-using existing dry wells that significantly reduce the overall cost of the structure. The method of testing the suitability of existing well for recharge is quite simple. The technology has the potential of scaling up across south India which has many dry and low-yielding tube wells.

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Towards DNA-based technological advancement in *Listeria monocytogenes* detection

Human listeriosis caused by *Listeria monocytogenes* (LM) is a serious public health concern. The disease leads to several health issues like abortion, stillbirth, septicaemia, meningitis and meningoencephalitis. The main targets are pregnant women, neonates, elderly or immunocompromised people[1]. LM has recently been reported as one of the most virulent pathogens with high rates of mortality (30%) and hospitalization (91%)[2]. It is a major cause of death (about 1600 cases) with an annual death rate of 255 in the United States alone. Such information for several countries including India is lacking. The ability of the pathogen to survive over a wide range of pH (4.3–9.8), salinity (up to 20% w/v) and temperature (0.5–45°C) makes it ubiquitous. It commonly contaminates raw food/products, and other food items through cross-contamination thus targeting humans. Therefore, the rapid detection of LM across diverse environments has great merit for food-processing industries, environmental quality control, and public health establishments.

Conventionally, detection and identification of LM involves culture-based methods that rely on the use of nutrient media for selective growth of the pathogen in targeted samples. Although morphological and biochemical confirmatory tests are sensitive, they are labour-intensive and time-consuming (5–7 days)[3]. Immunological techniques applied subsequently, viz. enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunofluorescent assay (ELFA) and immune-magnetic separation have detection limit of 10^5 cfu/ml (ref. 4). The immuno-detection techniques also suffer from certain disadvantages, viz. the quantity of sample, availability of pure antigens and cost-intensive chemicals that limit exploitation of such approaches for pathogen detection. Due to advancement in biotechnological tools/techniques, molecular biology (genomics/proteomics)-based approaches have become more popular, especially for food and medical microbiology, as they exploit pathogen differences at the genetic level. These methods can selectively amplify the *Listeria*-specific gene signals and are thus capable of detecting LM even at low copy numbers. This communication not

only offers an overview of the trends in LM detection, but highlights the conventional to recent breakthroughs, including future needs.

DNA hybridization is one of the simplest molecular methods. The nucleic acid probes derived from housekeeping and functional genes (16S rRNA, *inlA*, *plcA*, *prfA*, *iap*, etc.) of LM are used to detect the pathogen either by autoradiography (radiolabel) or using the appropriate substrate (enzyme label)[5]. Similar principle is also applicable to kits available commercially, e.g. GeneTrak®, Gene Quench®, AccuProbe®, etc. However, these techniques fail to track the target gene present at concentrations below 10^4 copies/µl and also do not utilize DNA amplification, thus limiting their sensitivity[5].

Nucleic acid amplification-based detection such as PCR (polymerase chain reaction) is one of the most promising approaches for pathogen detection. The earlier PCR methods applied to LM used the *hly* gene sequence, thereafter, 16S rRNA, 23S rRNA, *hlyA*, *plcA*, *plcB, actA*, *prfA*, *inlA*, *inlB*, *iap*, *dth18* and *lmo0733* genes were targeted[5,6]. Due to the genetic relatedness among *Listeria* species, the specific identification of LM through a single gene amplification is probably inadequate. Hence, alternatives to the classical PCR (multiplex PCR) have been developed to enhance pathogen detection. The co-amplification of a set of genes like *plcA*, *actA*, *hlyA* and *iap*, or *inlA*, *inlC* and *inlJ* has more accurately confirmed the presence of LM in a single PCR reaction[7–9].

It is crucial to estimate the concentration of cells in a sample and also distinguish dead from living cells for accurate detection of live, virulent LM. RNA is quickly eliminated after the death of an organism. Therefore, reverse transcriptase PCR (RT-PCR), targeting mRNA transcripts of genes like *hly*, *prfA* and *iap* is used for detecting the live LM cells[10,11]. However, the time for running agarose gel to analyse and detect the amplified products is a rate-limiting step for a large number of samples. Real-time quantitative PCR (qPCR) allows the amplicon observation directly, thus eliminating the post-PCR handling. This technique is also optimized for LM detection using *hly* as the target gene with a sensitivity of 100% (ref. 12). In addition, fluorescence resonance energy transfer (FRET) hybridization probes are used for the real-time assays with 26 h enrichment time. The method detects up to 1–5 cfu of LM per 25 g/ml of food samples within two days[13]. However, these techniques take several hours to identify LM as these involve the culturing of individual isolates before analysis. The techniques used so far provide scope for devising better alternatives which are affordable, rapid, easy to use and more sensitive for detecting low quantities of the pathogen in the suspected environment.

Recently, DNA-based biosensors (genosensor) have emerged as unique tools because of the simple fabrication process, quick response time, on-line and real-time detection of the pathogen. This technique is based on hybridization principle, and combines the use of biological receptors and physical and/or chemical transducers for the detection of target DNA in different environments. Several researchers have utilized this approach in genobiosensor development for detection of LM. For amperometric detection of the *hlyA* gene of LM, Ligaj *et al.*[14] described an electrochemical genosensor using carbon paste electrode and daunomycin. Farabullini *et al.*[15] used a disposable electrochemical low-density genosensor array to detect LM at nanomolar scale in less than an hour without any cross-interference. The electrochemical indicator toluidine blue has been used in a DNA electrochemical biosensor for the detection of LM in the range 1.0×10^{-7} to 8.0×10^{-5} M (ref. 16). In food products as well as organisms without labelling the target DNA, Wu *et al.*[17] reported a hybridization biosensor using $[Co(phen)_3](ClO_4)_3$ as the indicator of PCR-amplified products of *hlyA* gene. Sun *et al.*[18] used electrodeposition method for detecting specific LM gene (detection limit of 2.9×10^{-13} mol/l) ssDNA sequences by differential pulse voltammetric response of the methylene blue molecules accumulated on dsDNA molecules. Despite having several advantages, label-based sensing and amperometric approaches have certain limitations which mainly include potential interference to the response, leading to false current values.

Emergence of powerful techniques like electrochemical impedance spectroscopy (EIS) will lead to increased sensitivity of pathogen detection. This involves application of a small-amplitude sinusoidal excitation signal to the system and measuring the response. A DNA sensor, based on EIS, transduces the changes in the interfacial properties between the electrode and the electrolyte. In majority of the DNA sensors, the target DNA needs to be labelled with fluorophore, magnetic beads or an enzyme for pathogen detection. On the contrary, EIS-based DNA sensors do not require labelling, and thus are cost-effective, simple and have ease of miniaturization. For the rapid detection of LM, Kashish *et al.*[19] explored the feasibility of an EIS-based label-free genosensor with detection limit of 10^{-13} M. Although for LM detection molecular techniques are highly specific, pre-analytical steps such as sample collection, processing, handling time and temperature, and DNA extraction methods are the prerequisites, and adequate care is needed while studying the molecular microbial ecology of LM. Precise application of these steps improves downstream molecular applications, and thus the sensitivity and specificity of pathogen detection. Some studies report the significance of pre- and post-analytical steps in minimizing the errors in downstream analysis. Careful control measures in the laboratory and optimization of protocols will certainly ensure the quality of output and unwittingly reduce the chances of errors towards downstream analysis.

An ideal detection method should be simple, specific, sensitive, rapid, reproducible, efficient and cost-effective. Among various DNA-based detection techniques, the label-free impedimetric approach could be more appropriate for rapid and specific detection of LM. Despite these advancements in analytical techniques, the development and optimization of methods for LM detection from the samples having mixed bacterial population needs more emphasis. Rapid changes and innovation in technology invite attention towards the future and current genome/proteome-based detection tools/techniques to develop standard protocols and new technologies to limit the errors in the pre- and post-analytical steps. Scientific discussion on operating procedures and innovations could help these procedures to evolve as general tools in pathogen detection. Considering these aspects, the technique can be improved for devising commercial diagnostic kits for early detection of LM in different ecological niches, including clinical and environmental samples.

Therefore, an effort in this direction is likely to bring a significant breakthrough for diagnosis of listeriosis which is a neglected disease in various tropical countries, including India.

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A rapid and effective method for extraction of bacterial DNA from crude oil-contaminated soil

Crude oil contamination causes a major environmental problem. The crude oil changes the microbial population present in the soil, thereby affecting its properties[1]. The microbial community present in crude oil-contaminated soil has significant ecological importance. These microbes can be used as an effective tool for bioremediation. It has been well documented that soil contains the highest level of prokaryotic diversity than any other environment. One gram of soil can have more than 10 billion microorganisms comprising different species[2]. Two approaches can be used for isolating bacterial DNA from the soil. These are culturable and unculturable techniques, the latter being more effective as less than 1% of bacteria can be cultured in artificial media[3]. The basic steps in any unculturable technique comprises (a) processing of soil samples, (b) extraction of bacterial DNA directly from the soil, (c) downstream analysis of DNA which includes sequencing, phylogenetic analysis and other multivariate data analysis. The extraction of DNA is the most important step, as the purity of DNA determines its use for further downstream analysis. Sometimes contaminants such as humic acid precipitate with the DNA and hinder its amplification[4]. Various kits are available commercially for extraction of soil bacterial DNA, but only a few give good results with crude oil-contaminated soil[5]. Moreover, commercial DNA extraction kits are more expensive. Few reports have been documented on extraction of DNA from the soil using manual method[6]. However, no reports are available for the extraction of DNA from crude oil-contaminated soil. Therefore, we have developed a rapid, cost-effective method for extraction of pure DNA from crude oil-contaminated soil.

Soil samples were collected from the surrounding areas of Digboi oil refinery, i.e. Dubbs Para and Digboi Cenetary Park, Assam, India. They were processed by drying in a hot-air oven under 100°C for 2 h and sieved in a 2 mm mesh to separate the debris. Then 10 g of soil sample was weighed and 27 ml of extraction buffer (Tris-HCl, 100 mM EDTA, 1.5 M NaCl and 1% CTAB, pH 8) was added. Next, 1 ml of lysozyme (10 mg/ml) was added to this mixture (soil and extraction buffer) and incubated at 37°C for 2 h with continuous shaking at 150 rpm. Then 100 µl of proteinase K (10 mg/ml) and 3 ml of 20% SDS were added and the mixture was incubated at 65°C for 2 h in a water bath. After final incubation, the solution was centrifuged at 5981 rpm for 10 min. Subsequently, 8 ml of the supernatant was transferred to 50 ml centrifuge tube and equal volume of phenol : chloroform : isoamyl alcohol was added. The mixture was vortexed at maximum speed for 5 min. The aqueous phase was collected and 0.6 volumes of chilled ethanol was added and allowed to precipitate for 30 min. After precipitation, the solution was centrifuged at 8459 rpm for 10 min. The supernatant was discarded and pellet was dissolved in TE buffer. DNA was visualized by loading 4 µl in agarose gel (0.8%) for 50 min. The experiment was conducted in three replicates and repeated five times.