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A method of recovering the very low concentration of pathogens in river water by combining centrifugation and membrane filtration

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A R T I C L E I N F O

Method name:
Recovering the very low concentration of pathogens in river water by combining centrifugation with membrane filtration

Keywords:
Campylobacter
Risk
Environmental water

A B S T R A C T

Waterborne pathogens present major public health concerns because of the associated high mortality, morbidity and cost of treatment. Consumption of and contact with water contaminated by faeces is a significant risk factor for transmitting these organisms to humans. *Their* detection in a water sample is critical to ascertain potential risks to humans. They are relatively low in concentrations in surface waters, making their detection a challenge. *Campylobacter* is targeted here because it is one of the leading causes of enteric diseases globally, and consensus on the superiority of centrifugation over filtration, and vice versa, to recover *Campylobacter* spp. from river water samples for detection, has yet to be. Therefore, for this study river water sample was processed by combining both methods in a single set-up to concentrate *Campylobacter* spp. cells from water samples. This method of combining centrifugation and filtration can be expanded to other bacterial waterborne pathogens of public health importance.

- Concentrating cells by centrifugation (14,000 × g for 30 min) to collect the pellets, followed by membrane filtration (using 0.45 μm) of the supernatant to trap any remaining suspended cells, and then pooling both pellet and residue presents an effective method for obtaining a satisfactory quantitative recovery of waterborne pathogens, such as *Campylobacter* spp. from environmental waters.
- This is a critical need for quantitative microbial risk assessment studies.

Specifications table

Subject Area:	Agricultural and Biological Sciences
More specific subject area:	Microbiology
Method name:	Recovering the very low concentration of pathogens in river water by combining centrifugation with membrane filtration
Name and reference of original method:	Khan, I. U. H., Gannon, V., Loughborough, A., Jokinen, C., Kent, R., Koning, W., Lapen, D. R., Medeiros, D., Miller, J., Neumann, N., Phillips, R., Robertson, W., Schreier, H., Topp, E., van Bochove, E., & Edge, T. A. (2009). A methods comparison for the isolation and detection of thermophilic *Campylobacter* in agricultural watersheds. *Journal of Microbiological Methods, 79*(3), 307–313. 10.1016/j.mimet.2009.09.024
Resource availability:	NA

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*Method details

To spot the very low concentration of pathogens in water, an initial step of concentration is necessary for their detection. It is following concentrating the target organism from a large volume of water that effective detection of pathogens can be achieved [1–3]. This method is based on concentration by centrifugation and membrane filtration and subsequent pooling of cells in pellet and membrane filter residue [4]. The idea is to trap any suspended bacterial cells that were not pelleted [5]. *Campylobacter* is targeted here because it is one of the leading causes of enteric diseases globally, and consensus on the superiority of centrifugation over filtration, and vice versa, to recover *Campylobacter* spp. from river water samples for detection, has yet to be. To target *Campylobacter*, following, the recovery step, highly selective cultivation approaches was applied. However, this method of recovering pathogens from water samples can be expanded to other bacterial waterborne pathogens of public health importance, including *Clostridium, Salmonella, Staphylococcus, and Escherichia coli*, that occur in large volumes of water at relatively low concentrations. River water samples are collected at the field by immersing sterile containers 5 cm beneath the river's surface. The collected samples should be stored in ice cooler boxes and transported to the lab for analysis. Membrane filters with 0.45 μm pore sizes effectively retain *Campylobacter* cells from water samples [6,7]. Therefore, a 0.45 μm pore size membrane filter was used in this study.

Apparatus

500 ml,Carl Roth - Centrifuge bottles made of PPCO with leak-tight closure cap.
Beckman Coulter Avent® J-E 14,000 centrifuge (rotter JSE 06A08)
0.45 μm pore size (47 mm diameter) filter membrane (Millipore)
OXOID Anaerobic Jars (2.5 L)
Pall - Vacuum/Pressure Pump 230 V, 50/60 Hz,CE
Pall - FILTER FUNNEL MAGNETIC W/O LID 300ML PPS
1,5 m Tubing for Vacuum Filtration Setup, 8 mm ID, 12 mm OD, Wall Thickness 2.5 mm
Vortex mixer
Heratherm Microbiological Incubator
CampyGen, Gas Gen Oxoid sachets

Procedure

1. Place 500 mL of river water samples in a centrifuge bottle and centrifuge using a Beckman Coulter Avent® J-E 14,000 centrifuge (rotter JSE 06A08) at 14,000 × g for 30 min.
2. Separate the supernatant from the pellet by decanting it into a sterile conical flask. Collect the supernatant.
3. To the pellet in the centrifuge bottle, add 10 mL of sterile distilled water and vortex to mix.
4. Filter the collected supernatant through a 0.45 μm pore size (47 mm diameter) filter membrane (Millipore) to trap any suspended Campylobacter that was not pelleted. Collect the membrane filter containing the residue.
5. Using sterile scissors, chop the membrane filter into the pellet mixture.
6. Place the pellet and chopped membrane filter mixture into a 100 mL conical flask containing 90 mL of Bolton Broth.
7. Place the flask in an OXOID Anaerobic Jars (2.5 L), and place CampyGen compact around the flask to create a microaerobic environment.
8. Place the Anaerobic Jar in the incubator and incubate at 4 ˚C for 48 h so that growth can be observed as a pellicle at the surface of the Broth (Fig. 1).
9. Pipette 100 μL of enrichment broth culture by placing the pipette tip just below the surface layer of the broth media.
10. Spread the liquid culture across the whole surface of a modified cefoperazone charcoal deoxycholate agar (mCCDA) plate by rubbing back and forth using a glass spreader while rotating the plate four times in different directions.

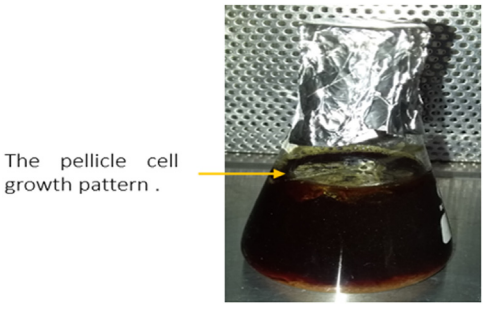

Fig. 1. A photograph off 100 mL flask with pellicle cell growth pattern observed after enrichment of samples in Bolton Broth medium Growth Characterised by the formation a thick layer covering the surface of the media.

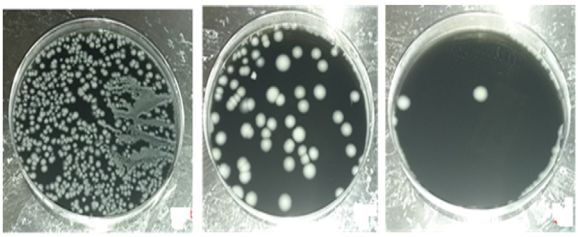

Fig. 2. A photograph of mCCDAagar plates demonstrating morphology of the colonies obtained by grab sampling at least 500 mL of river water samples and concentrating by centrif ugation combined with membrane filtration method. Round moist white cream/y ellow colonies were observed.

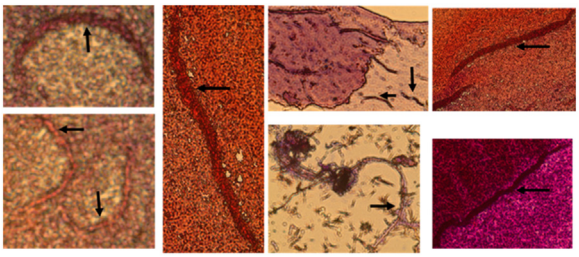

Fig. 3. Microscopic image of Gram stain for randomly selected colonies from mCCDA plates, obtained by culturing *Campylobacter* cells from river water samples processed by using centrifugation combined with membrane filtration. Magnification:1000.

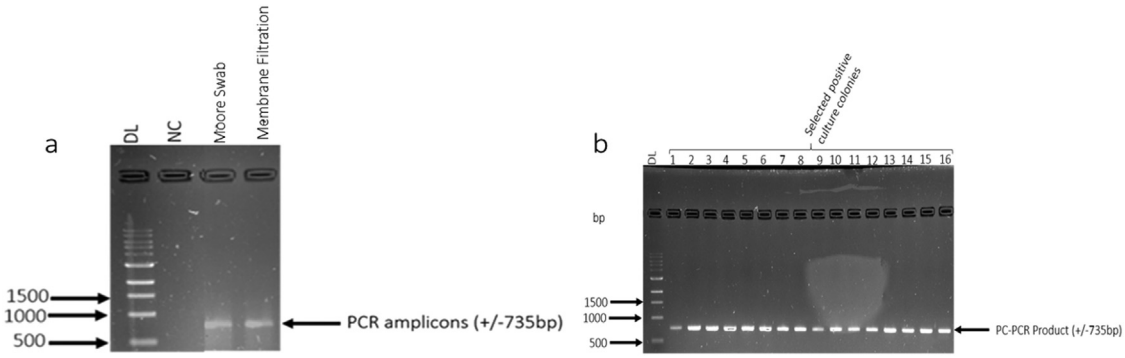

Fig. 4. Photograph of UV illuminated, stained 1% Agarose gel display ing visible bands observed for river colonies by (a) recovered from river water by combining centrif ugation with membrane filtration (b) freshly prepared C. *jejuni* colonies. From Left to Right, Lane DL: Quickoad Purple 1 kb DNA ladder. Lane NC: Negative Control. The *Campylobacter Jejuni* HIP400F and HIP113R primer was used.

11. Place plates in OXOID Anaerobic Jars (2.5 L), with CampyGen compact, placed one in between two plates.
12. Incubate the Anaerobic Jar at 42 ℃ for 48 h.
13. Observe the plates for round moist white cream/yellow colonies of Campylobacter (Fig. 2).

Method validation

Confirmed colonies are indeed *Campylobacter* by observing pink rods, spiral, and curved through Gram staining (Fig. 3) and colony polymerase chain reaction (PCR). Gel electrophoresis of the colony PCR amplicons should show amplicons of the same size as amplicons obtained from PCR assay of freshly prepared colonies *Campylobacter jejuni* as positive controls. (Fig. 4). The sequence obtained for the PCR product showed at least 98% homology with *C. jejuni* as evaluated by NCBI BLAST. In addition, the method from this study, combining centrifugation and membrane filtration, compared to the Moore swab method, yielded more positive samples, i.e. 47% and 11%, respectively (Fig. 5). The Moore swab method has been used for years by global public health experts to recover enteric pathogens from water.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

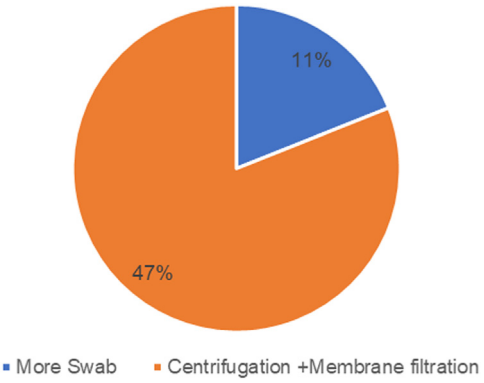

Fig. 5. Comparison of Campylobacter spp. detection rates by recovering in water by combining centrifugation with membrane filtration and by the Moore swab method. The percentage of the total number of collected samples that tested positive for Campylobacter spp is higher using centrifugation combined with membrane filtration of grab water samples than using the Moore Swab sampling method.

Data availability

Data will be made available on request.

Acknowledgements

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