ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD

DISCUSSION PAPER

THE ISOLATION OF *CAMPYLOBACTER SPP* FROM FOOD AND ENVIRONMENTAL SAMPLES

In October 2009 the Food Standards Agency published its UK-wide survey of *Campylobacter* and *Salmonella* contamination of chicken at retail sale. The ACMSF Surveillance Working Group (SWG) had informed Members about the discrepancy it observed in the survey results when they considered a draft version of the survey report in March 2009. Although the survey contractors provided clarifications on the issues the SWG raised, the SWG informed Members at the September 2009 ACMSF meeting that they will draft a commentary paper on surveillance of retail chicken in the UK outlining some issues that had arisen from recent FSA *Campylobacter* surveys relating to methods and approach. The SWG indicated that the Committee would be asked to consider the commentary paper before it is forwarded to the FSA. The aim of the paper is to provide recommendations for the FSA to consider for their future surveillance work.

Members are invited to:

- Approve the attached paper (ACM/994a) The Isolation of *Campylobacter spp*. from food and environmental samples) and agree that it is forwarded to the FSA

Secretariat
September 2010

ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD

DISCUSSION PAPER

The isolation of *Campylobacter* spp. from food and environmental samples

Background

There is a public health and policy need to reduce the risk to consumers from foods contaminated with *Campylobacter* and particularly the threat from chicken meat. Effective food surveillance tools must be part of any such strategy, particularly in respect of the presence of *Campylobacter* spp. and subsequent assessment of the efficacy of intervention measures aimed at reducing the public health threat from these important zoonotic pathogens. This requires the appropriate choice of sample type, source and frequency of sampling, quantity, method of sampling (whole, excision, rinse, swab etc.) and test sample size (weight, volume, area). The combined sampling methodology must be able to reliably recover and deliver *Campylobacter* cells present to the isolation procedure.

Sensitive and specific methods are required for the isolation of *Campylobacter* spp. The choice of media and incubation conditions (atmosphere, temperature and time) and use of enrichment stages should be appropriate for the sample type. The isolation of *Campylobacter* spp. from food and environmental samples is usually achieved by enrichment culture in one of a number of selective broths (Table 1) followed by plating onto selective agar, which is often Modified Charcoal Cefoperozone Deoxycholate agar (mCCDA) in the UK. The information presented in this brief report is to inform discussion and does not constitute a recommendation for any particular medium and/or isolation protocol.

Isolation of *Campylobacter* spp. from food samples is time consuming and difficult, as the past two national surveys on contamination of raw poultry on retail sale have illustrated. Surveillance of chicken provides a good model for the problems inherent in the isolation of *Campylobacter* spp. from foods and the techniques and isolation strategies that need to be applied are very different from samples such as water (Figure 1).

Table 1: **Commonly used enrichment broths for the isolation of *Campylobacter* spp***

Broth	Selective agents used Concentrations in 1 litre of medium	Incubation regimes
Bolton	20mg Cefoperazone, Vancomycin and Trimethoprim, 50mg Cycloheximide	37^{o}C for 4h, 41.5^{o}C for 44h
****mBolton**	20mg Cefoperazone, Vancomycin and Trimethoprim, 10mg Amphotericin B	37^{o}C for 4h, 41.5^{o}C for 44h
Preston	5mg Trimethoprim, 5mg Rifampicin, 2500iu Polymyxin B and 5mg Amphotericin B	41.5^{o}C for 48h
****mExeter**	10mg Trimethoprim, 5 mg Rifampicin, 2500iu Polymyxin B, 15mg Cefoperazone and 2mg Amphotericin B	37^{o}C for 48h

* Taken from the PhD thesis of Dr Lisa Williams, University of Bristol
**These media have been modified from their original formulation

Technical difficulties in the isolation of *Campylobacter* from food and environmental samples

There are three critical factors that govern the success of isolation techniques:

- The numbers of *Campylobacter* spp. present in /on the test sample
- The physiological state of the *Campylobacter* cells
- The size and nature of the competing microbial population

Achieving a balance between allowing *Campylobacter* cells to recover and suppressing competing microorganisms: The two sample types, chicken and water, chosen to illustrate the usefulness of isolation strategies in this document, represent different ends of a sample spectrum. With water, the *Campylobacter* population can be highly injured by exposure to the extra-intestinal environment (see below) and be present in low numbers. In addition, the competing bacterial population can be low in number. With chicken, the competing bacterial population can be large and can contain bacteria such as *Pseudomonas* and *Proteus* spp., the growth of which has been shown to affect the survival of *Campylobacter* spp. in a mixed culture.

For samples such as water, the critical factor in isolating *Campylobacter* is to allow the recovery of damaged cells of this pathogen. Thus, in addition to suppressing the growth of competing microorganisms, a successful enrichment medium should allow the recovery and subsequent growth of the target *Campylobacter* population These bacteria can be highly sensitive to the extra-intestinal environment. One important manifestation of this is that cells of *Campylobacter* in food and environmental samples will often be “sub-lethally injured” as a consequence of exposure to common environmental conditions such as low temperature, drying, UV light etc. Sub-lethal injury is manifested by sensitivity to selective agents in media and incubation conditions to which non-injured cells are normally tolerant. Thus, injured cells of *C. jejuni* are sensitive to the antibiotic rifampicin and the commonly used incubation temperature of 42ºC. Injured cells will recover resistance to selective agents and tolerance of elevated incubation temperatures if they are incubated in non-selective media at 37ºC.

However, with samples like chicken, which have a large and mixed bacterial population, the critical factor in the isolation of *Campylobacter*, is to suppress the growth of competing microorganisms.

Recovery techniques successful with water, such as withholding some selective agents and incubating samples at 37ºC, do not work as well with chicken. This strategy allows competing bacteria to grow and inhibit the growth of *Campylobacter* spp. This is illustrated by Fig. 1, which shows data from past work (Humphrey, unpublished), which compared isolation rates from water and chicken samples. As Figure 1 shows, with water samples, a delay in the addition of the selective agents used in Exeter broth (Table 1) for up to eight hours increased isolation rates. However, a delay of only four hours reduced isolation rates from chicken samples from ~80% to ~55%.

Selective agents used in media for isolating *Campylobacter* spp: A range of selective agents is added to enrichment broths to try and limit the growth of competing bacteria (Table 1), with varying success. Table 2 shows a comparison of isolation rates of *Campylobacter* spp. from naturally contaminated chicken and environmental samples using three commonly used enrichment broths. With both sample types, mExeter medium was significantly better than either mBolton or Preston broths.

Table 2: Isolation of *Campylobacter* spp. from retail chickens and environmental samples in relation to the enrichment medium used*

Enrichment medium	Number of positive samples (% positive)	
	Environmental samples (n = 144)	Retail chicken samples (n = 40)
mExeter	99 (68)	30 (75)
mBolton	85 (59)	28 (70)
Preston	76 (53)	22 (55)

* Taken from the PhD thesis of Dr Lisa Williams, University of Bristol

There is a need for an effective and reproducible method for the isolation of *Campylobacter* from chicken

It is clear from the results of the last two FSA-funded surveys of *Campylobacter* contamination of chicken at retail, that laboratories are experiencing difficulties in the isolation of the target bacteria. The impetus in the last few years has been for the development of rapid methods for the isolation/detection of *Campylobacter* and there is a danger that simple basic criteria have been forgotten: the most important of which are that the method used must be both sensitive and specific.

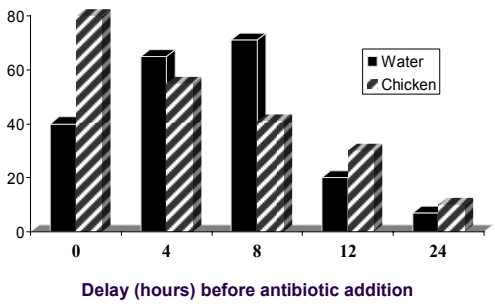
It also appears that there is a lack of authoritative information of how basic laboratory protocols affect the isolation of *Campylobacter*. Thus, there is a need for the following simple questions to be answered:

- What is the best method for sampling chicken products?
- What is the best test sample type to use for chicken products?
- Is there a reliable method for acquiring e.g. by concentration (immunological means), the target organisms from the test sample or the enrichment media to improve their detection if present?
- Which are the best isolation media for *Campylobacter* in/on chicken samples?
- How long is it safe to leave chicken test samples e.g. carcass rinses, before antibiotics are added?
- What are the optimum incubation conditions for chicken test samples including carcass rinses?

Are new methods required?

It is likely that current isolation protocols will not allow the identification of the maximum number of *Campylobacter*-positive samples from food and environmental samples and new strategies may be required to improve isolation methods.

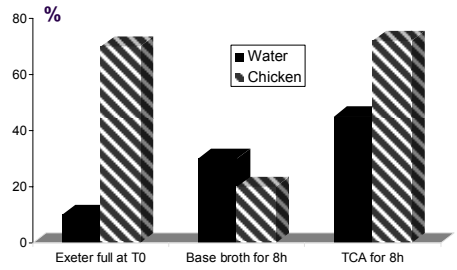
Fig. 1: Delaying antibiotic addition reduces *Campylobacter* isolation rates from chicken samples



Published information suggests that further studies to develop protocols based on modification of isolation media and associated incubation conditions including combinations of these would be worthwhile. It should be remembered that current methods involve the use of only a very small

aliquot of an enriched sample and generally, only those target cells that are freely suspended will be sub-cultured (others that are attached to surfaces or debris will remain in the bulk enriched volume). Methods to maximise the availability of target cells for selective isolation on agar media could usefully be explored.

Fig. 2: A strategy for isolation of damaged *Campylobacter*



A specific area of enrichment media use that could be worth further investigation is two-step addition of selective agents. Past work by the chairman of the Surveillance Group (TJH) examined the five selective agents used in Exeter broth (Table 1). These studies showed that sub-lethally injured cells of *C. jejuni* were not sensitive to three of the antibiotics used in that medium, Trimethoprim, Cefoperazone and Amphotericin. If these are added to samples at the beginning of incubation, the growth of contaminants is suppressed for approximately eight hours at 37ºC. During this time, injured cells of *Campylobacter* recovered and regained resistance to the remaining two "Exeter" antibiotics,

Rifampicin and Polymyxin B. These can then be added without the risk of isolation rates being reduced. Naturally contaminated chicken and water samples were used to compare isolation rates of *Campylobacter* spp. when samples were inoculated:

- Directly into full mExeter both (Exeter full at T0),
- Into base broth only, which was incubated for eight hours at 37ºC until all the antibiotics were added
- Into base broth containing Trimethoprim, Cefoperazone and Amphotericin, which was incubated for eight hours (TCA for eight hours) at 37ºC until Rifampicin and Polymyxin B were added.

The results presented in Figure 2 show that two-stage addition of selective agents, where the growth of contaminants was initially suppressed using Trimethoprim, Cefoperazone and Amphotericin, significantly improved isolation rates of *Campylobacter* from water. Isolation rates from chickens were unaffected by this protocol.

Research needs

The Food Standards Agency should give consideration to funding work which examines the basic requirements for the successful isolation of *Campylobacter* spp. from food and environmental samples. Such work should include a re-examination of sampling methods, handling of test samples and importantly, each of the steps employed in isolation methods. Current published material should be used to inform and guide such work.

The Surveillance Working Group
August 2010