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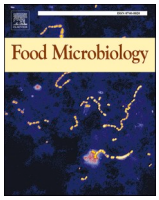

Repeated cross-sectional study identifies differing risk factors associated with microbial contamination in common food products in the United Kingdom

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ABSTRACT

All foods carry microbes, many of which are harmless, but foods can also carry pathogens and/or microbial indicators of contamination. Limited information exists on the co-occurrence of microbes of food safety concern and the factors associated with their presence. Here, a population-based repeated cross-sectional design was used to determine the prevalence and co-occurrence of *Escherichia coli, Klebsiella* spp., *Salmonella* spp. and *Vibrio* spp. in key food commodities - chicken, pork, prawns, salmon and leafy greens. Prevalence in 1,369 food samples for these four target bacterial genera/species varied, while 25.6% of all samples had at least two of the target bacteria and eight different combinations of bacteria were observed as co-occurrence profiles in raw prawns. Imported frozen chicken was 6.4 times more likely to contain *Salmonella* than domestic chicken, and imported salmon was 5.5 times more likely to be contaminated with *E. coli*. Seasonality was significantly associated with *E. coli* and *Klebsiella* spp. contamination in leafy greens, with higher detection in summer and autumn. Moreover, the odds of *Klebsiella* spp. contamination were higher in summer in chicken and pork samples. These results provide insight on the bacterial species present on foods at retail, and identify factors associated with the presence of individual bacteria, which are highly relevant for food safety risk assessments and the design of surveillance programmes.

1. Introduction

Food products are an important vehicle of gastrointestinal pathogen transmission, with foodborne illness associated with a high economic and health cost (DEFRA, 2021; Holland and Mahmoudzadeh, 2020; WHO, 2015); in the United Kingdom, it is estimated there are 2.4 million cases of foodborne illness annually (Daniel et al., 2022). While there are many bacteria which are known foodborne pathogens, such as *Salmonella* and *Vibrio*, the food safety threat represented by other bacteria in the food chain is less well understood.

Non-typhoidal *Salmonella* spp. is a major foodborne pathogen, estimated to cost £212 million annually (Daniel et al., 2022); in recent years, *Salmonella* spp. outbreaks have been associated with frozen breaded chicken products, raw and ready-to-eat bean sprouts and seeds, and eggs (ECDC-EFSA, 2021; Jørgensen et al., 2022; Pijnacker et al., 2019; Sadler-Reeves et al., 2016). The epidemiology of *Salmonella* has changed over time and is monitored in surveillance programmes and identified as part of source attribution outbreak investigations (Besser, 2018; ECDC, 2021; Gov. of Canada, 2019a), yet its survival in the food chain and ability to contaminate a wide assortment of food products continues to challenge food safety interventions (Besser, 2018).

Another organism causing foodborne outbreaks, primarily through the consumption of seafood, is *Vibrio* (Baker-Austin et al., 2018, 2020). Pathogenic *Vibrio* can cause mild to severe gastroenteritis, bacteraemia, and in some cases wound infections. *Vibrio* cases are associated with a higher incidence in summer months (Baker-Austin et al., 2018). With trends of increasing seafood consumption and the sensitivity of marine environments to climate change, *Vibrio* is posing an increasing risk to food safety but with limited surveillance, the number of vibriosis

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infections is uncertain (Baker-Austin et al., 2017; Garcia et al., 2009). Recently, a genomic investigation of *Vibrio* in prawns sold at retail in the UK identified a high level of contamination, with 46% of prawns carrying *Vibrio*. However, the subtypes of *Vibrio* contaminating the prawns did not present an immediate risk to food safety. The study also revealed diverse populations of *Vibrio* on the prawns which has implications for outbreak traceback and source attribution (Janecko et al., 2021).

Klebsiella is an opportunistic pathogen found in many environments such as soil and the intestines of animals and humans (Hartantyo et al., 2020). *Klebsiella pneumoniae* is a major cause of both nosocomial and community-acquired infections, and multidrug resistant *K. pneumoniae* is listed as a critical priority antimicrobial-resistant pathogen by the WHO (WHO, 2019). Multidrug-resistant and extended spectrum beta-lactamase (ESBL)-producing *K. pneumoniae* have been reported in healthy populations (Huynh et al., 2020) and recently, studies reported multidrug-resistant *K. pneumoniae* in foods of animal and plant origin (Rodrigues et al., 2022; Theocharidi et al., 2022). The role of *Klebsiella* spp. from food as a risk to human health is not known, but as *Klebsiella* strains can be hypervirulent and/or associated with antimicrobial resistance (AMR) genes (Hartantyo et al., 2020; Lv et al., 2020; Wan et al., 2021; WHO, 2019), it warrants consideration as a potential foodborne threat.

In terms of AMR, *E. coli* is the predominant choice as a Gram-negative sentinel microorganism in surveillance programmes monitoring AMR trends in the food chain. Its presence is also used as an indicator organism by the food industry and regulatory bodies to assess hygiene and possible presence of other pathogens (HPA, 2009).

A general feature of most surveillance programmes is that the prevalence of all organisms of interest are reported separately, with little information available on the co-occurrence of organisms of interest within a single sample (ECDC, 2017, 2019; EFSA, 2008; EFSA et al., 2019; FDA, 2022; Gov. of Canada, 2019a,b). This lack of knowledge on co-occurrence limits our understanding of the potential for gene flow of AMR or virulence genes between bacterial species, as well as the overall food safety risk presented by particular foods (Le Roux and Blokesch, 2018; Rossi et al., 2014). For instance, elevated levels of *E. coli* were correlated with *Salmonella* detection in chicken and pork and similarly, higher *E. coli* levels were linked to the presence of verotoxigenic *E. coli* and *Salmonella* in leafy greens (Ceuppens et al., 2015; Ghafir et al., 2008). Adding to the complexity, each food type has different properties and undergoes different processing techniques that may affect microbial risks. For example, the surface structure of leafy greens and the ability of pathogens to internalise into the inner leaf can render washing procedures ineffective (Doan et al., 2020; Grivokostopoulos et al., 2022; Turner et al., 2019) and present food safety risks which are different from those affecting foods of animal origin, such as cross-contamination in abattoirs (Boubendir et al., 2021; Zeng et al., 2021). In prawn production, the process of peeling shells is associated with cross-contamination of *Vibrio parahaemolyticus* between prawns (Xiao et al., 2018) and in poultry abattoirs, disinfection between flock processing was identified as a contamination point (Zeng et al., 2021), identifying processing as critical control points to the microbiological safety of the end food product.

To gain insights into the degree of co-occurrence of contamination with pathogens and indicator organisms, we targeted four bacterial genera/species important to food safety to assess contamination of key food products. The objectives of this investigation were to 1) determine the prevalence of three pathogens – *Salmonella*, *Vibrio* and *Klebsiella* – and the contamination indicator organism *E. coli* in key food products at retail in the UK; 2) determine co-occurrence detection of these targeted bacterial genera/species, and 3) to identify risk factors associated with recovery of the targeted bacterial genera/species.

2. Materials and methods

2.1. Study design and sample collection

Sample collection was designed to evaluate key bacterial genera/species that contaminate retail food products at the nearest point to consumer exposure. A population-stratified and market share-weighted repeated cross-sectional study was designed and conducted in the county of Norfolk in the UK between May 2018 and November 2019. This was achieved using Office of National Statistics 2011 census figures for Norfolk and city population records to determine the population distribution in urban and rural areas and define four strata based on population sizes: stratum-1, rural (10% of the county population), stratum-2 semi-urban (10% of the county population), stratum-3 urban (55% of the county population) and stratum-4 large city (Norwich, 25% of the county population) (ONS., 2018). The number of planned sample collections per stratum were weighted by population size and designed for 42 sampling trips, with stratum-1 and -2 receiving four visits each, stratum-3 receiving 23 visits and stratum-4 receiving 11 visits within the sampling period (Supplementary Table 1).

A list of available stores in the region was created and categorised into chain, independent and butcher shops. Market share information (Kantar, 2018) was used to determine the frequency of visits per store name; overall, the proportion of chain store:butcher shop/independent store visits was 75%:25%. The number of sampling trips within each stratum was set to be representative of estimated consumer foot traffic by population and to reflect the exposure by the general population of Norfolk (Supplementary Table 1). If samples for a particular commodity were not available in a store, a similar store type from a back-up list was visited to complete the sample set for each food commodity per sampling trip.

Sample types were chosen based on the Family Foods 2015 consumption data (DEFRA, 2017) to incorporate highly consumed products. Commodities selected were raw chicken, raw pork, raw salmon, raw prawns, cooked prawns and packaged leafy greens. Each commodity sub-type was selected by the most available to consumers at the time of store visit (Supplementary Table 2).

At each store visit, two samples per commodity were chosen with at least two days remaining within the 'best before' or 'use-by' date, and when available, one sample of each commodity was to be domestically produced and one imported from outside the UK. For meat sub-types, conventionally raised samples were predominantly chosen as they were the most common, although when available some niche market products (organic, free-range, ethically raised, halal, kosher) were also selected. For seafood (salmon and prawns) no preference was given to aquacultured or wild caught. To eliminate cross contamination post-collection, each food sample was packaged individually into a zipper-sealed bag and transported to the laboratory in temperature-controlled coolers to maintain the temperature at time of purchase. Once at the laboratory, each sample was photographed, and product information available on packaging or from shop staff was recorded into pre-defined categories and each sample was stored in a refrigerated environment until sample processing. If information was not available, it was defined as 'unlabelled' in the analysis.

2.2. Sample processing and preparation

Sample processing was conducted within 24 h of purchasing at the Quadram Institute Bioscience laboratory, Norwich, UK. Each food sample package was wiped with disinfectant prior to being opened using sterilised instruments to minimise cross-contamination. Sterilised instruments were used to weigh out a single 100 g portion of each food sample, and aseptically transferred into sterile filtered stomacher bags. Specifically, chicken pieces included skin on and skinless, bone-in or boneless; pork included bone-in and boneless, prawns included different levels of exoskeleton dressing and salmon was either skin on or skinless.

All samples were treated equally by mass. Samples purchased frozen were placed onto the laboratory bench no more than 2 h before processing to allow sufficient thawing for processing. All sample types were homogenised in 225 mL of buffered peptone water (BPW) (Southern Group Laboratory (SGL), Corby, UK) at 100 rpm for 30 s (Seward stomacher 400C laboratory blender, Worthing, UK) and incubated at 37 °C for 24 h ± 3 h. For samples with bone-in or shell-on, homogenisation was performed manually by massaging the stomacher bags for 2 min. Additionally, for prawn and salmon samples, 100 g of sample was homogenised in 225 mL alkaline peptone water (APW) (Southern Group Laboratory, Corby, UK) in preparation for *Vibrio* detection and incubated at 28 °C for 24 h ± 3 h.

2.3. Escherichia coli microbiological detection

All food samples were tested for *E. coli* by inoculating 50 mL of incubated BPW into 50 mL of *E. coli* enrichment broth double concentration (EC 2x) (ThermoFisher Diagnostics, Rochford, UK) and further incubated at 42 °C for 24 h ± 3 h. All subsequent incubations were conducted at 37 °C for 24 h ± 3 h. A 10 μL loopful of incubated broth was plated onto Eosin methylene blue agar (EMB) (Sigma Aldrich, Haverhill, UK) and incubated. Up to four colonies expressing typical *E. coli* morphology (dark maroon colony with or without metallic sheen) were sub-cultured onto MacConkey agar (ThermoFisher Diagnostics, Rochford, UK) to assess for the lactose fermentation characteristics of *E. coli* and further plated onto tryptic soy agar (Trafalgar Scientific Ltd., Leicester, UK) to conduct biochemical confirmation using Simmon's citrate agar (Sigma Aldrich, Haverhill, UK) and Remel™ indole spot reagent test (Fisher Scientific, Loughborough, UK). Up to four confirmed *E. coli* isolates per sample were preserved.

Isolates of *E. coli* and all subsequent bacteria isolated in this study were stored at −70 °C in 1 mL of Brucella broth +17.5% glycerol (ThermoFisher Diagnostics, Rochford, UK).

2.4. Klebsiella spp. microbiological detection

Detection for *Klebsiella* was conducted on all food samples collected by inoculating 10 μL loopful of incubated BPW onto MacConkey agar and incubating at 37 °C for 24 h ± 3 h. Two typical mucoid colonies were sub-cultured onto secondary MacConkey agar for purification, followed by inoculating onto tryptic soy agar. Each incubation was at 37 °C for 24 h ± 3 h. Confirmation of *Klebsiella* spp. was conducted using Christensen's urea agar (Fisher Scientific, Loughborough, UK) and Simmon's citrate agar. Up to two confirmed *Klebsiella* spp. isolates per sample were preserved.

2.5. Salmonella spp. microbiological detection

Detection of *Salmonella* spp. was conducted for each sample type using an adapted ISO 6579–1:2017 method (ISO, 2017a). Briefly, using the incubated BPW, a parallel selective enrichment was conducted with 1 mL of BPW into 10 mL of Muller-Kaufman tetrathionate broth (MKTTn) (Fisher Scientific, Loughborough, UK), incubated at 37 °C for 24 h ± 3 h and 0.1 mL of BPW inoculated onto modified semi-solid Rappaport Vassiliadis (MSRV) plate (Sigma Aldrich, Haverhill, UK) with an incubation of 42 °C for 24 h–48 h. Once incubated, a 10 μL loopful of presumptive *Salmonella* growth on MSRV and a 10 μL loopful of MKTTn broth was plated onto xylose-lysine-deoxycholate agar and brilliance *Salmonella* bi-plates (ThermoFisher Diagnostics, Rochford, UK). All subsequent incubations were at 37 °C for 24 h ± 3 h. Presumptive positive colonies were sub-cultured onto MacConkey agar to assess lactose fermentation and purify culture, followed by a further sub-culture of presumptive positive colonies onto tryptic soy agar. Confirmation of *Salmonella* spp. was conducted using Christensen's urea agar, Remel™ indole spot reagent test and Difco™ agglutination test using *Salmonella* O poly A-I&Vi antiserum (Fisher Scientific, Loughborough, UK). Up to eight isolates of *Salmonella* spp. per sample were preserved.

2.6. Vibrio spp. microbiological detection

Prawn and salmon food samples were tested for *Vibrio* spp. using a previously described adaptation (Janecko et al., 2021) of the ISO 21872–1:2017 method (ISO, 2017b). In brief, using a 10 μL loop, the top portion of the incubated APW broth was skimmed and inoculated onto thiosulfate citrate bile salt (TCBS) agar (Oxoid, Basingstoke, UK). All incubations were conducted at 28 °C for 18–24 h ± 3 h. One typical blue/green colony morphology and one yellow colony with specified 2–3 mm diameter were sub-cultured onto Columbia blood agar with 5% sheep's blood (Trafalgar Scientific, Leicester, UK). Confirmation of *Vibrio* spp. was conducted using the oxidase production test (Oxoid, Basingstoke, UK), while phenotypic disk diffusion test using O129 discs was noted (Themo Fisher Scientific, Loughborough, UK). Up to two isolates of *Vibrio* spp. per sample were preserved.

2.7. Statistical analyses

Food samples were considered positive for a particular target organism if at least one isolate of that target bacterium was identified. Descriptive analysis using food sample metadata and detection of each target bacterium was conducted using R version 4.1.2 (R Core Team, 2020). Prevalences of each target bacterium were analysed by food commodity (chicken, leafy greens, pork, prawns-raw, prawns-cooked, salmon) as were associations with potential explanatory variables of interest such as season of purchase (winter – December to February, spring – March to May, summer – June to August and autumn – September to November), food origin (imported or domestically produced), store type (chain or independent/butcher) and food presentation at the time of purchase (chilled or frozen). Additional variables were also considered for leafy greens and raw prawns, such as the wash status (washed or unwashed) and conventionally or aquacultured prawn species, respectively. Two-tailed 95% confidence intervals (CIs) for the proportions of each food commodity testing positive for each of the target bacteria were estimated using the "goodman" method available through the function MultinomCI of DescTools version 0.99.4 R package (Signorell et al., 2021). Fisher's Exact Test from the rstatix's v0.5.0 R package (Kassambara, 2020) was used to compare proportions of the target bacteria presence between categories (e.g., imported vs domestic) for each potential explanatory variable (e.g., food origin) described above by food commodity. A Bonferroni correction was applied to account for multiple hypothesis testing. To assess the association of factors with recovery of *E. coli, Klebsiella* spp., *Salmonella* spp. and *Vibrio* spp. within each food commodity, we used contingency tables (2 × 2) of the presence/absence of the bacterium with the factor of interest (e.g., domestic chicken meat vs. imported chicken meat). The odds ratios (ORs) were calculated from the contingency tables using unconditional maximum likelihood estimation along with two-tailed 95% CIs by normal approximation, which are available in the oddsratio (contingency table, method = "wald") function of the Epitools version 0.5.10.1 R package (Aragon et al., 2020). To analyse co-occurrence of target bacteria, a bacterial profile was defined as the combination of target bacteria identified within an individual sample. Prevalence estimates of single genus/species and multiple genera/species bacterial profiles were calculated with two-tailed 95% CIs and plotted across food commodities and explanatory variables of interest using the ggplot2 version 3.3.5 R package (Wickham et al., 2016).

3. Results

3.1. Sample collection

Between May 2018 and November 2019, a total of 203 food retail outlets were visited and 1,369 food samples purchased. This included

139 chain stores, 45 butcher shops and 19 independent shops (greengrocers, fish mongers, independent grocers). For each commodity, a variety of available food sample cuts were purchased as chilled and frozen. A total of 311 raw chicken, 311 raw pork, 157 raw salmon, 217 raw prawns, 62 cooked prawns and 311 leafy greens were purchased, and sub-categorisations were recorded (Supplementary Table 3; Supplementary Figure 1).

Commodity samples were purchased according to availability with domestic products comprising 68.8% of chicken, 79.1% of pork, 50.0% of cooked prawns, 37.6% of leafy greens and 33.8% of salmon samples. Remaining proportions consisted of imported food samples and those of unknown origin. Most raw prawns (92.6%) were imported.

3.2. Detection of E. coli

E. coli was detected in the vast majority of raw chicken (98.7%) and raw pork (89.7%) samples and at a lower frequency in leafy greens (40.5%), salmon (29.9%), and prawns [37.8% - raw; 29.0% - cooked] (Table 1). While *E. coli* detection varied between commodities, no significant difference in *E. coli* detection was observed for all commodities between chain vs butchers/independent shops and chilled vs frozen samples (Fig. 1; Supplementary Table 4). When analysing proportions of *E. coli* based on origin, the odds of *E. coli* detection was significantly higher in imported salmon ($p < 0.01$; OR = 5.54, 95% CI: 2.05–14.97) than domestic or unlabelled products ($p < 0.05$; OR = 5.79, 95% CI: 2.17–15.39) (Fig. 2A; Supplementary Table 5).

Packages of washed leafy greens had a significantly higher prevalence ($p < 0.05$; OR = 3.32, 95% CI: 1.37–8.04) of *E. coli* than unlabelled wash status packages of leafy greens (Supplementary Table 6; Fig. 3A), but there was no difference between washed and unwashed leafy greens.

In prawns, the frequency of *E. coli* detection varied between production systems and species. Conventionally aquacultured black tiger raw prawns were more likely to carry *E. coli* than conventionally aquacultured raw king prawns ($p < 0.05$; OR = 3.49, 95% CI: 1.60–7.61) (Supplementary Table 7; Fig. 3B). *E. coli* was detected in 25.0% (2/8) of organically aquacultured and 8.3% (1/12) of wild caught raw prawns (Table 2). The prevalence of *E. coli* contamination ranged from 7.7% (Argentine red prawns) to 63.4% (black tiger prawns) between the three prawn species represented at retail (Table 2).

For leafy greens, significantly higher proportions of samples tested positive for *E. coli* in summer than in winter or spring ($p < 0.001$) and higher in autumn than spring or winter ($p < 0.05$) (Fig. 2B; Supplementary Table 8). Seasonal differences were not seen for *E. coli* contamination in any other commodity.

3.3. Detection of Klebsiella

Klebsiella was detected in all commodities, with prevalence ranging from 3.2% to 33.2% (Table 1). In the ready-to-eat sample types, *Klebsiella* contamination was 3.2% and 38.9% in cooked prawns and washed leafy greens, respectively. In raw products, the prevalence was 7.6% on salmon, 21.9% on chicken, 25.5% on unwashed leafy greens, 32.5% on pork and 33.2% on raw prawns.

There were no significant variations in *Klebsiella* contamination of commodities when comparing store type (chain vs butcher/independent), food presentation (chilled vs frozen) at the time of purchase or origin of product (Figs. 1 and 2). In leafy greens, no significant differences were observed by wash status (Fig. 3A; Supplementary Table 6). The detection of *Klebsiella* was significantly higher ($p < 0.05$) in summer than winter for chicken and pork, and significantly higher in summer and autumn than winter for leafy greens (Fig. 2B; Supplementary Table 8). Season of purchase was not associated with seafood testing positive for *Klebsiella* (Fig. 2B).

3.4. Detection of Salmonella

Salmonella was detected in chicken (9.6%), pork (1.3%) and raw prawns (3.7%). Within chicken, *Salmonella* contaminated 5.3% (12/225) of chilled and 20.9% (18/86) of frozen chicken (Fig. 1B). Of the frozen chicken sample types purchased, 96.5% (83/86) were imported (Fig. 2A). All frozen chicken contaminated with *Salmonella* (n = 18) were imported and packed in the same country represented by nine suppliers. A significantly higher proportion of imported chicken tested positive for *Salmonella* than did domestically produced chicken ($p < 0.001$; OR = 6.38, 95% CI: 2.8–14.55) (Supplementary Table 5).

Four samples of pork were positive for *Salmonella,* of which all samples were domestically produced: chilled pork chop cuts purchased from chains (n = 3) and a butcher shop (n = 1). Of the 3.7% (n = 8) of raw prawns positive for *Salmonella* spp., seven were black tiger prawns (*Penaeus monodon*) of which five were conventionally aquacultured (Table 2). No seasonal differences were observed for *Salmonella* detection (Fig. 2B). There were no significant variations in *Salmonella* detected in tested sample types and food presentation (chilled vs. frozen) at the time of purchase, with the exception of frozen chicken that was significantly higher than chilled ($p < 0.0001$; OR = 4.70, 95% CI: 2.15–10.26) (Fig. 1B).

3.5. Detection of Vibrio

In the seafood tested, 60.4% of raw prawns, 8.1% of cooked prawns and 2.5% of salmon were contaminated with *Vibrio* (Table 1). A high proportion of *Vibrio* contamination was observed in raw prawns purchased from chain stores (61.5%), while in butcher/independent stores a high proportion of contamination was observed for cooked prawns (66.7%) (Fig. 1A); no significant differences in *Vibrio* contamination were detected in any seafood sample type when comparing food presentation (chilled vs frozen) (Fig. 1B). The contamination of raw prawns with *Vibrio* varied between imported and unknown origin with no domestic raw prawns available for purchase (Fig. 2A). Of the labelled raw prawn packages, *Vibrio* contaminated 62.8% (118/188) of conventionally aquacultured prawns, 100% (8/8) of organically aquacultured prawns and 8.3% (1/12) of wild-caught prawns (Table 2). Of the three

Table 1
Proportion of samples containing *E. coli, Klebsiella* spp., *Salmonella* spp., and *Vibrio* spp. in each commodity purchased in Norfolk, UK between May 2018 and November 2019.

Sample type	Proportion of samples			
	E. coli % (n)	*Klebsiella* % (n)	*Salmonella* % (n)	*Vibrio* % (n)
Chicken (n = 311)	98.7 (307)	21.9 (68)	9.6 (30)	n/a
Pork (n = 311)	89 .7 (279)	32.5 (101)	1.3 (4)	n/a
Leafy greens[a] (n = 311)	40.5 (126)	33.1 (103)	0 (0)	n/a
Salmon (n = 157)	29.9 (47)	7.6 (12)	0 (0)	2.5 (4)
Prawns – raw (n = 217)	37.8 (82)	33.2 (72)	3.7 (8)	60.4 (131)
Prawns – cooked (n = 62)	29.0 (18)	3.2 (2)	0 (0)	8.1 (5)

n/a: not applicable; testing of *Vibrio* was not conducted for land-based food commodities.
[a] Leafy greens include whole head of lettuce, single leaf pre-cut packages, mixed leaf pre-cut packages.

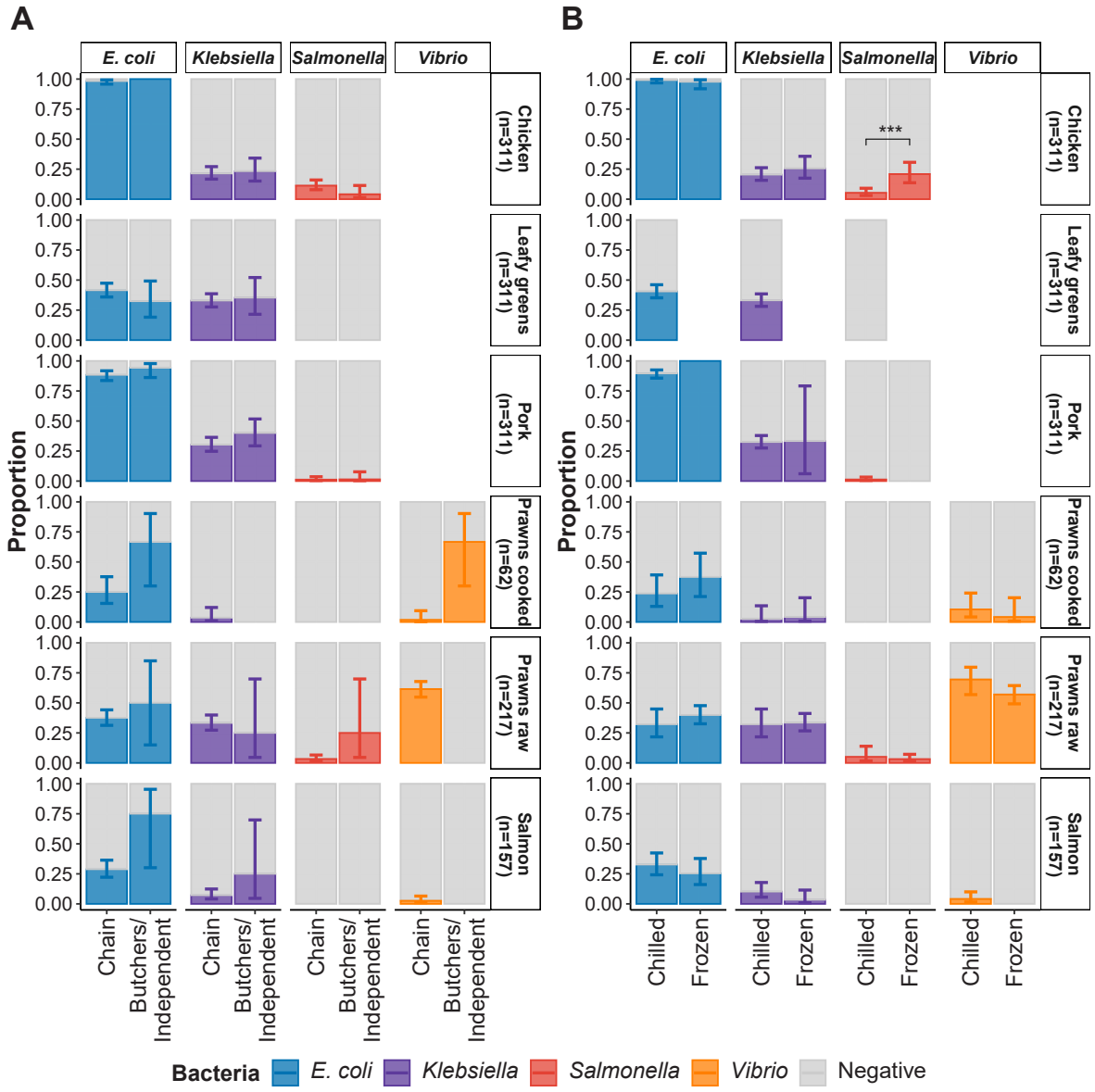

Fig. 1. Proportion of chicken, leafy greens, pork, prawns (raw and cooked) and salmon contaminated with each targeted bacterium by (A) store type and (B) food sample presentation (chilled or frozen) at the time of purchase, with 95% confidence intervals presented. Statistical significance is presented by *** for $p \leq 0.001$.

main prawn species represented at retail in the UK, only two species were found in the aquaculture conventional production type and no significant difference in *Vibrio* contamination was observed between these species (Fig. 3B; Supplementary Table 7). No seasonal differences were observed across the seafood commodities for *Vibrio* detection (Fig. 2B).

3.6. Co-occurrence of target bacteria

The most common co-occurrence profile overall was the *E. coli* – *Klebsiella* spp. combination (16.9%; 232/1369). In raw prawns, the most common co-occurrence profiles included the *Klebsiella* – *Vibrio* (12.9%; 28/217) combination and the *E. coli* – *Vibrio* (11.5%; 25/217) combination (Fig. 4; Supplementary Table 9). The numbers of profiles differed between commodities with one co-occurrence profile observed in leafy greens, two different co-occurrence profiles in cooked prawns, three each in chicken, pork and salmon, and eight different co-occurrence profiles in raw prawns with five samples (5/217) containing all four tested bacteria (*E. coli* – *Klebsiella* – *Salmonella* – *Vibrio)* (Fig. 4; Supplementary Table 9). *Salmonella* was the only bacterium always detected in combination with other bacteria, predominantly *E. coli* or *E. coli* and *Klebsiella*. In raw prawns, *Salmonella* was also detected in combination with *Vibrio*, and with *E. coli* – *Vibrio*, in individual samples (Fig. 4; Supplementary Table 9).

4. Discussion

Foods are covered in diverse populations of microorganisms that can include non-pathogenic microbes, opportunistic pathogens and pathogens. In this study, we targeted foodborne pathogens and indicators of contamination in key food products as part of a population-based survey in 2018 and 2019. Using these data, we determined the prevalence of the individual target bacteria, the degree of co-occurrence detection of these bacteria, and identified factors associated with the presence of these organisms.

E. coli has been used as an indicator organism for assessing hygiene practices in the food chain and biological hazard risk assessments (Allende et al., 2018; Ghafir et al., 2008; HPA, 2009). The high detection

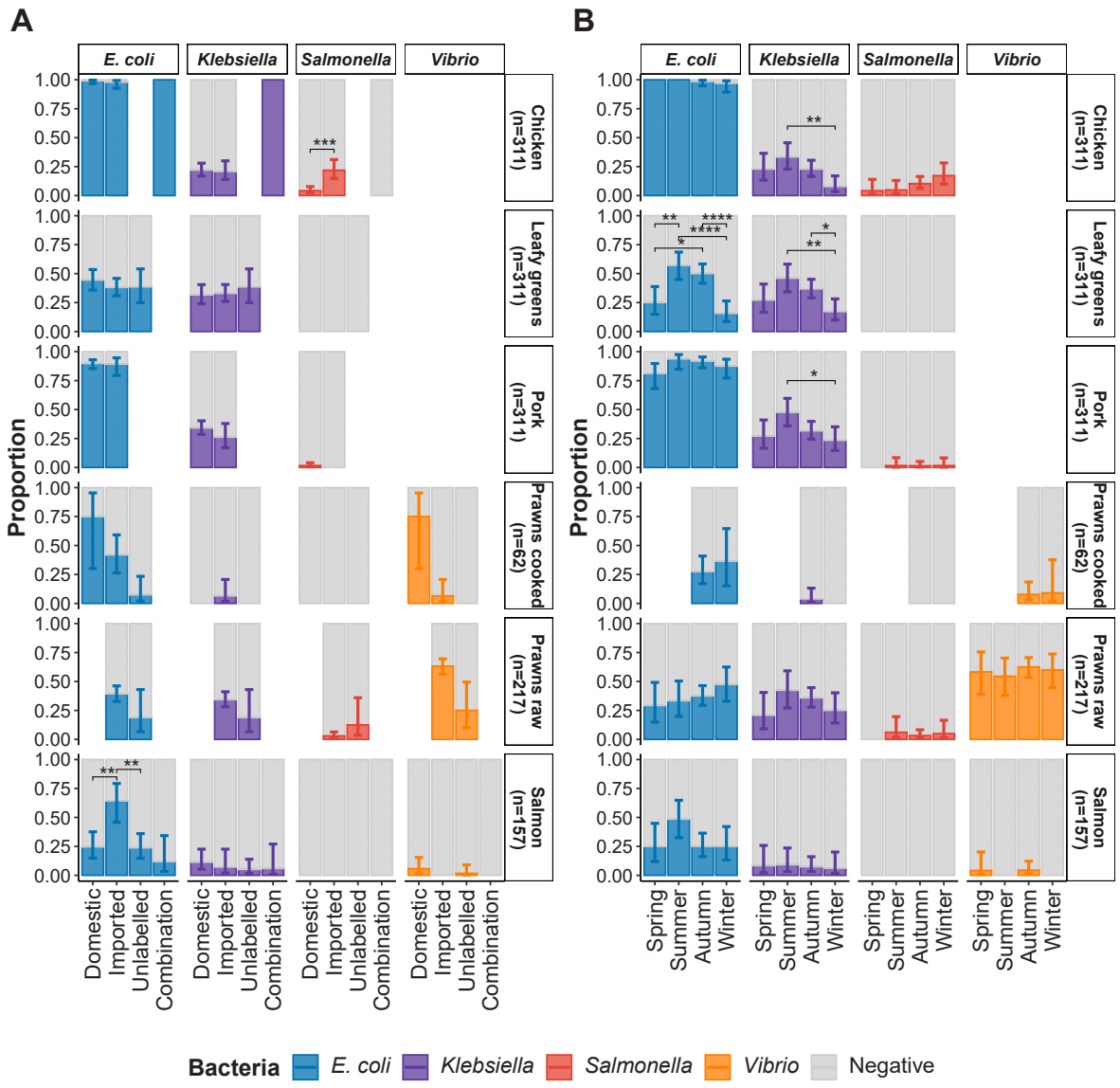

Fig. 2. Proportion of chicken, leafy greens, pork, prawns (raw and cooked) and salmon contaminated with each targeted bacterium by (A) origin of product designation (domestic, imported, unlabelled, combination) and (B) by seasons from samples collected between May 2018 to November 2019, with 95% confidence intervals presented. Statistical significance is presented by *, **, ***, **** for $p < 0.05$, $p \leq 0.01$, $p \leq 0.001$ and $p \leq 0.0001$, respectively.

of *E. coli* (98.7%) in chilled and frozen chicken in our study was equivalent to the high detection levels in frozen breaded chicken products sampled within the same time frame in the UK (Jørgensen et al., 2022), Canadian chilled chicken meat (Gov. of Canada, 2020) and Belgian poultry meats in 2008 (Ghafir et al., 2008). This level of detection in chicken meat is relatively higher than has been reported in other regions of the world such as Brazil, Sri Lanka, Thailand and Cambodia (Crecencio et al., 2020; Kulasooriya et al., 2019; Trongjit et al., 2016), where the prevalence of *E. coli* in chicken ranged from 10 to 74%. Similar trends were found for *E. coli* contamination of pork, where our study identified high instance (89.7%) while others were considerably lower ranging between 18 and 58% (Ghafir et al., 2008; Gov. of Canada, 2020; Trongjit et al., 2016). The differences observed may be due to heterogeneity in the sampling design, study objectives and microbiological methods employed. The starting sample type differs among studies and surveillance systems and typically ranges from swabs of carcasses (Ghafir et al., 2008; Trongjit et al., 2016) or meat sample portions of 25 g (Jørgensen et al., 2022) to 50 g portion of meat (FDA, 2022). In our study, a larger portion of starting sample was used (100 g), and multiple colonies of presumptive *E. coli* were tested, thereby optimising detection. Factors associated with *E. coli* detection were assessed in our study, and no significant differences were observed between store types, chilled vs frozen meats, nor was there a seasonal prevalence trend in meat and seafood samples. Similarly, no seasonal trend was observed in modelling climate impact on *E. coli* detection in retail chicken and pork sold at retail in Canada (Smith et al., 2019). Additionally, temperature storage risk factors (chilled vs frozen) of poultry meat did not significantly contribute to *E. coli* contamination differences in other studies (Eltai et al., 2020; Kulasooriya et al., 2019). However, seasonal differences were observed in leafy greens in our study, where summer and autumn months yielded significantly higher sample prevalences of *E. coli* than in winter and spring months. It is unclear what other factors may be influencing this observation, but possible contributing factors may be the growing conditions, water sources, and/or weather patterns in different growing regions throughout the year.

In leafy greens, *E. coli* is an indicator of faecal contamination by soil or water sources (Allard et al., 2019; Allende et al., 2018; Benjamin et al., 2013). Our study detected 40.5% of samples contaminated with *E. coli,* which was higher than studies investigating on-farm,

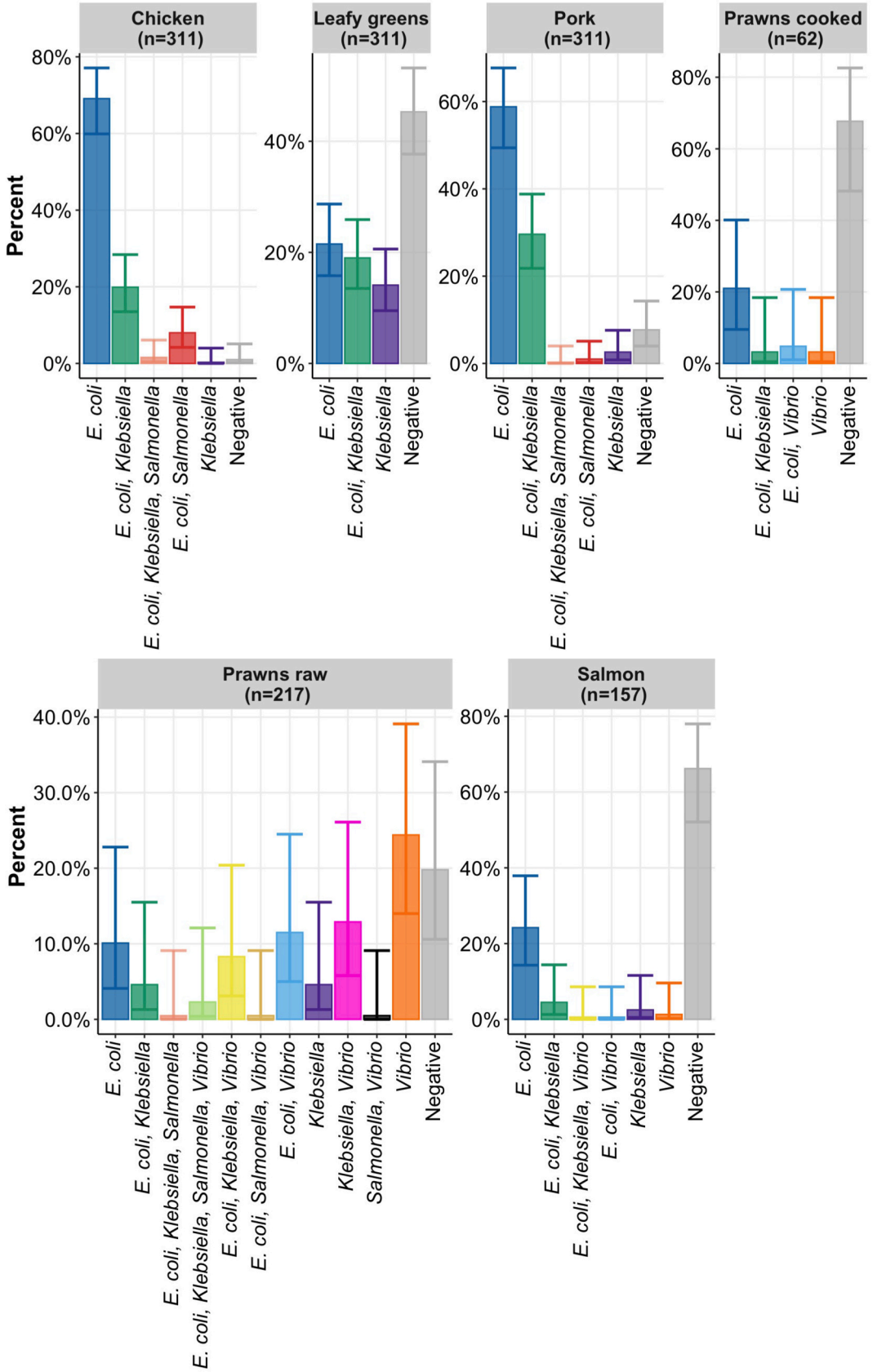

Fig. 3. (A) Proportion of *E. coli* and *Klebsiella* spp. detected in leafy greens purchased at retail containing label information on the wash status and (B) proportions of targeted bacteria detected on raw prawns by conventionally aquacultured prawn species with 95% confidence intervals presented. Statistical significance is presented by *, **, for $p < 0.05$ and $p \leq 0.01$, respectively.

Table 2
Number and proportion of target bacteria detected in raw prawn purchases at retail by production type and prawn species.

Production type	Prawn species	Proportion of prawn species n (%)			
		E. coli	*Klebsiella*	*Salmonella*	*Vibrio*
Aquaculture conventional (n = 188)	King prawns (*Litopenaeus vannamei*) (n = 154)	53 (34.4)	54 (35.1)	1 (0.6)	92 (59.7)
	Black tiger prawns (*Penaeus monodon*) (n = 34)	22 (64.7)	12 (35.3)	5 (14.7)	26 (76.5)
Aquaculture organic (n = 8)	King prawns (*Litopenaeus vannamei*) (n = 4)	1 (25.0)	2 (50.0)	0 (0)	4 (100)
	Black tiger prawns (*Penaeus monodon*) (n = 4)	1 (25.0)	1 (25.0)	0 (0)	4 (100)
Wild caught (n = 12)	Argentine red prawns (*Pleoticus muelleri*) (n = 12)	1 (8.3)	1 (8.3)	0 (0)	1 (8.3)
Unknown (n = 9)	King prawns (*Litopenaeus vannamei*) (n = 4)	0 (0)	0 (0)	0 (0)	2 (50.0)
	Black tiger prawns (*Penaeus monodon*) (n = 3)	3 (100)	2 (66.7)	2 (66.7)	1 (33.3)
	Argentine red prawns (*Pleoticus muelleri*) (n = 1)	0 (0)	0 (0)	0 (0)	1 (100)
	Unknown species (n = 1)	1 (100)	0 (0)	0 (0)	0 (0)

post-production and at point of sale in other regions with prevalences ranging from too low to detect to 23.8% (Allard et al., 2019; Jongman and Korsten, 2016; Richter, 2022; Zhang et al., 2018). The type and variety of leafy green products in our study differed from others, which could account in part for this difference. Packages of mixed-leaf greens in our study were from different farms and in some cases different countries, further complicating an understanding of the risk factors leading to *E. coli* contamination. The topography of leaves, and in particular the venation of leaves may be a protective factor in *E. coli* survivability by preventing *E. coli* removal by wash and disinfection exposures (Doan et al., 2020). We observed significant differences in *E. coli* detection between washed packages of leafy greens and unlabelled packages, with significantly higher *E. coli* presence in washed products in our sample set. The mechanism behind this is unclear, as the unlabelled packages could represent washed or unwashed greens, although previous outbreak investigations involving leafy greens found leaf structure, irrigation water supply, moisture levels and environment affected the presence of indicator organisms and foodborne pathogens (Belias et al., 2020; Doan et al., 2020; Grivokostopoulos et al., 2022; Smolinski et al., 2018; Turner et al., 2019). In a large multi-state investigation of leafy greens, no difference in levels of *E. coli* were observed between ready-to-eat (washed) and unprocessed (unwashed) leafy greens (Zhang et al., 2018), and our results support this finding.

Klebsiella was detected in all commodities tested with a range of prevalences between 3.2% and 33.2%. The 21.9% of chicken samples in this study contaminated with *Klebsiella* is consistent with previous studies, where *Klebsiella* was found in between 13.8% and 65.7% present of raw fresh poultry at retail (Guo et al., 2016; Hartantyo et al., 2020; Rodrigues et al., 2022; Theocharidi et al., 2022). Raw seafood products investigated by Guo et al. observed a lower (8.2%) prevalence of *Klebsiella* than that reported in raw prawns (33.2%) in this study. The presence of *Klebsiella* detected in leafy greens in this study was within the previously reported prevalence in lettuce (53%) and prepared salads (20.7%) (Hartantyo et al., 2020; Rodrigues et al., 2022). In this study, risk factor analysis did not identify food presentation, store type or origin of product for any commodity as associated with *Klebsiella* presence. The same significant seasonal difference observed for both *E. coli* and *Klebsiella* detected in leafy green products in this study suggests *E. coli* may be an appropriate sentinel organism for *Klebsiella*

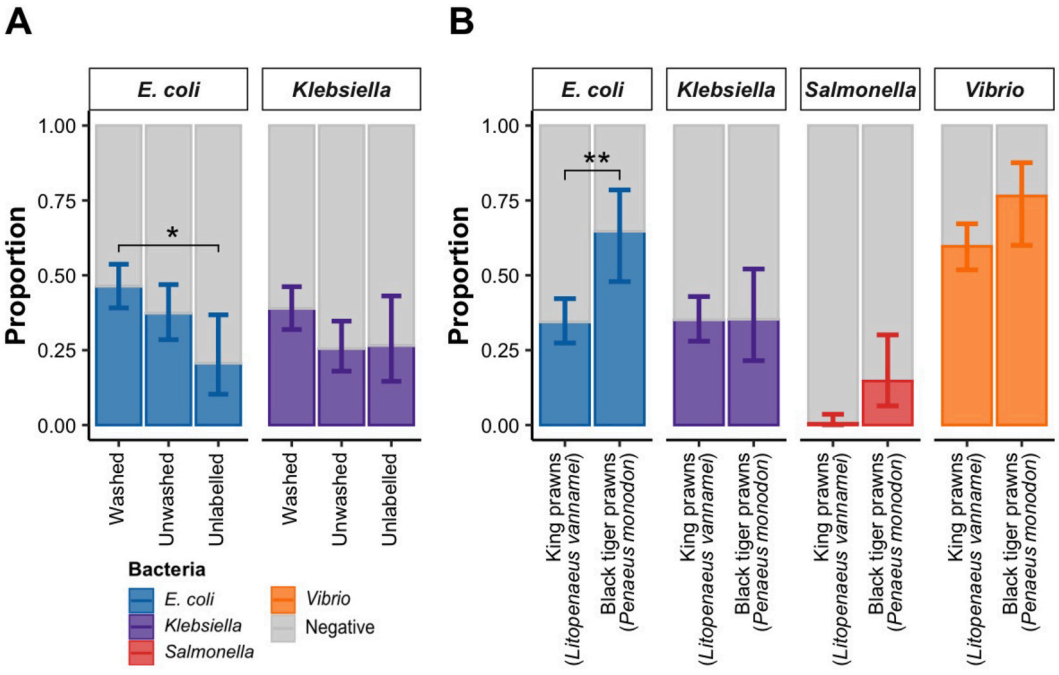

Fig. 4. Distribution and percentage of contamination with single and multi-bacteria profiles in each food sample type tested with 95% confidence intervals.

contamination of leafy greens, although further investigation and confirmation would be needed. Food as a vehicle of *Klebsiella* transmission has been reported in a nosocomial setting through hospital catering kitchens, but the direction of transmission and source of the original contamination was not clear (Calbo et al., 2011). The relatively high contamination observed in this study and possibility of hypervirulent and multidrug resistant strains in ready-to-eat foods such as leafy greens and other fresh produce warrant further investigation.

We report *Salmonella* contamination in chicken (9.6%), pork (1.3%) and raw prawns (3.7%) with no detection in leafy greens or salmon. Recovery rates of *Salmonella* in chilled retail chicken of surveillance programs were relatively higher in Canada with 20% and 27.6% reported, while USA (9.1%) and European (7.7%) surveillance systems reported *Salmonella* prevalence more closely aligned with our findings (EFSA-ECDC, 2021; Gov. of Canada 2019; Gov. of Canada, 2020; NARMS, 2017). The prevalence of *Salmonella* on raw chicken samples in our study was higher (9.6%) than a previous survey conducted in the UK reporting 5.7% (FSA, 2001). This may be due to the different chicken cuts included in our study, region tested, or time period of samples collected. Where imported products and domestic products could be compared, *Salmonella* was detected more often in imported frozen chicken in our study. Similar findings were reported recently in the UK, linking salmonellosis cases to imported frozen breaded chicken products (ECDC-EFSA, 2021; FSA, 2001; Jørgensen et al., 2022). Although different store types have been identified as a risk factor for higher foodborne pathogen prevalence in foods in some studies (Jørgensen et al., 2021; Su et al., 2021), store type was not associated with *Salmonella* detection in the five commodities of this study. Seasonal fluctuations of *Salmonella* were not detected for the five commodities tested, which aligns to findings of models utilising temperature, precipitation and seasons in Canadian pork, chicken and beef agrifood chains from farm to retail (Smith et al., 2019). The absence of *Salmonella* in leafy greens of this study is consistent with the low levels of 0.05% found in previous studies (Zhang et al., 2018). Seasonal trends were also investigated in a quantitative microbial risk assessment on leafy green production with very small effects observed, albeit for the indicator organism of *E. coli* (Allende et al., 2018).

The presence of *Vibrio* detection in raw prawns (60.4%), cooked prawns (8.1%) and salmon (2.5%) was not substantially different from what we previously reported in a subset of the prawn samples (Janecko et al., 2021). Investigations in prawn producing regions found the prevalence of *Vibrio* there ranged from 87% to 100% (Letchumanan et al., 2015; Tan et al., 2020; Yen et al., 2020), while in importing regions, such as Germany, lower levels (57.5%) (Vu et al., 2018) similar to what we observed were reported. Marine environments in which prawns are either aquacultured or wild caught are sensitive to climate change, and specifically water temperature fluctuation is a key factor to the abundance of *Vibrio* in marine waters (Baker-Austin et al., 2012, 2017). Season of purchase was not a risk factor for higher presence of *Vibrio* detected in raw prawns in this study, although season of purchase may not reflect season of harvest since prawns are typically flash frozen shortly after harvest and can be stored long-term as frozen (FAO and WHO, 2020; Hamilton and Chen, 2018). Furthermore, a risk assessment conducted for foodborne pathogens present on prawns implicated processing stages as major risk factors for contamination rather than pond or estuary conditions (Hamilton and Chen, 2018). Aquaculture of prawns is based on two dominant species (king prawns/white-leg prawns and black tiger prawns); here, we report 60.5% and 75.6% of white-leg and black tiger prawns, respectively, contaminated with *Vibrio*, similar to a previous study (Yen et al., 2020). There may be a difference in the regions these two species are raised or the conditions in which these are raised to explain the difference in contamination presence between the two species. Although the numbers were not suitable for formal comparison, wild caught prawns were all the same species (Argentine red prawn) which was caught in Atlantic waters rather than Pacific or Indian ocean regions. Further study would be needed to study risk factors for the presence of pathogens and indicator bacteria associated with prawn species, production type and country of origin.

Vibrio was detected at a low abundance (2.5%) in salmon fillets with all contaminated samples collected from chain stores as chilled products, most of which were domestically produced. A previous study assessing the prevalence of *Vibrio* from retail and restaurants also identified low levels of contamination, albeit higher (9.0% and 15%) than those reported in this study (Xie et al., 2019). The general low detection of *Vibrio* in salmon samples in comparison to prawns included in our study may be due to the cold marine waters in which salmon are aquacultured or caught, as a higher prevalence of *Vibrio* (39.6%) in fish raised in warmer waters were observed by Dewi et al. (2022). The genetic diversity and AMR in *Vibrio* populations characterised in retail prawns previously (Janecko et al., 2021) and the prevalence of *Vibrio* detected in this study warrant further inclusion of *Vibrio* as a microbial hazard into food risk assessments, a sentinel species of climate change effects on food systems and public health surveillance systems.

We report extensive detection of co-occurrence of four target bacteria in highly consumed food commodities with $\geq$30% of samples containing at least two target bacteria in chicken, pork and raw prawns. Observational studies and surveillance systems investigating foodborne pathogens and indicator organisms typically report individual organism prevalence of detection independently without indicating multi-bacteria profiles per sample (Benjamin et al., 2013; FDA, 2022; FSA, 2001; Gov. of Canada, 2019a,b; Pijnacker et al., 2019). In studies where multiple bacterial species were investigated, elevated *E. coli* levels were correlated with the presence of *Salmonella* in beef, pork and poultry and indicated *Campylobacter* presence in poultry (Ghafir et al., 2008). In our study, 9.6% of chicken and 1.3% of pork contained *E. coli* with *Salmonella* in the co-occurrence profile either alone or with another target bacterium. In a smaller sample set of raw chicken products in Sri Lanka, 30% of samples contained an *E. coli* – *Campylobacter* profile (Kulasooriya et al., 2019). Co-occurrence of *Salmonella* and *Campylobacter* has previously been reported to be 3% in fresh and frozen chicken in the UK (FSA, 2001) and more recently, 8.8% of frozen breaded chicken products were found to be contaminated with *Salmonella* and were associated with elevated levels of *E. coli* identified through enumeration (Jørgensen et al., 2022). Whilst our study used a method that indicates the presence of bacteria rather than the overall bacterial load, our study similarly demonstrates the presence of multiple pathogens within widely consumed food products. Particularly, in our study, *Salmonella* was the only bacterium that was always detected with other bacteria, primarily with *E. coli* and to a lesser extent with other organisms (Fig. 4; Supplementary Table 9). However, given the low *Salmonella* recovery and very high recovery of *E. coli* (Table 1), formal statistical association analysis of *Salmonella* recovery with other bacteria was not possible. Raw prawns contained the widest range of co-occurrence profiles with eight different combinations; overall, 41% of tested raw prawn samples contained two or more targeted bacteria.

This study demonstrated the diverse foodborne pathogens present on widely varying foods of animal and plant origin; as part of the microbial community on foods, this presents an opportunity for horizontal gene exchange between microbes of potentially harmful traits, such as virulence or AMR (Rossi et al., 2014). Although this was beyond the scope of the present study, future investigation into this would inform our knowledge of food safety risks.

5. Conclusions

Microbes do not exist on foods in isolation. The objectives of this investigation were to identify microbial threats in food commodities widely consumed in the UK using a repeated cross sectional study design and describe the co-occurrence of four target microbes in food commodities available to consumers in a specific region of the UK. Risk factor analysis identified significant associations of *E. coli* presence with imported salmon, *Salmonella* presence with imported frozen chicken,

E. coli seasonality in leafy greens and seasonality of *Klebsiella* detected in chicken, pork and leafy greens. The role of seasonality is not fully understood as a driver of foodborne illness incidence for the commodities and target bacteria tested. Factors such as human behaviour and food consumption habits may further affect contamination of some commodities and target bacteria combinations (Smith et al., 2019). In addition, there are other multiple and dynamic factors influencing microbial populations on foods throughout the entire food system, including those on farm, in abattoirs and during processing. These include animal husbandry considerations including antimicrobial use, and biosecurity and sanitation practices, factors which may be region-dependent and can change over time. Therefore, the risk factors identified here may not necessarily reflect those of other food types, time periods or geographical origins, highlighting the importance of ongoing microbial surveillance across the food chain. The presence of *E. coli* and *Klebsiella* in all food types tested, both individually and with other target bacteria, as well as the overall diversity of multi-bacteria profiles warrant further investigation; this greater knowledge of microbial dynamics on food matrices will be key to understanding the overall microbial risk from these food commodities in the UK and can be compared with other foods and with foods from other regions. The inclusion of co-occurrence reporting in surveillance programmes would build on a food safety foundation and provide wider breadth to a One Health approach to risk assessment.

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Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2022.104196.

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