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Prioritisation of Hazards Associated With Probiotics Used in Aquaculture by an e-Delphi Approach: Consideration of the Farmer, Consumer, Crop Species and Environment

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ABSTRACT

Probiotics are increasingly applied in aquaculture, yet these products can pose safety concerns, including the presence of contaminant microorganisms. A prioritisation of the potential hazards associated with these products would support appropriate risk assessment and help to guide the development of effective regulations and targeted enforcement. An e-Delphi approach was taken to capture expert opinions on the relative importance of potential hazards linked to the intrinsic properties of probiotic products and their application in aquaculture. In total, 17 potential hazards were identified by the panel of experienced researchers, and this curated list was prioritised by the 12 panellists according to their implications for the safety of four key receptors, specifically the farmer, consumer, cultured crop species and wider environment. After three rounds of iterative evaluation by the panel using a 0–100 scoring scheme, contamination with viable pathogenic microorganisms and horizontal transfer of antimicrobial resistance (AMR) and virulence genes were perceived to be the three most important hazards, ranking in the top four hazards for each receptor. There was significant agreement in hazard rankings by the panellists and this increased throughout the exercise, as confirmed by increasing Kendall's coefficient of concordance (W) values. This is the first study to prioritise the hazards associated with the intrinsic properties of probiotic products used in aquaculture. One limitation was panel composition, which may have constrained the diversity of perspectives and future exercises could consider a broader membership. Still, the findings provide evidence to support regulators in the development of interventions that address the most serious safety concerns, which will support the safe use of probiotics in aquaculture, helping to unlock their full potential.

1 | Introduction

Probiotics in aquaculture are commonly applied in feed or to the water to enhance growth performance, disease resistance, and water quality [1–3]. However, there is increasing concern for the safety of probiotic products, even those available commercially in some countries [4–7], with recent investigations highlighting issues such as the presence of microbial contaminants, including pathogens [6, 8, 9]. There are also risks that viable non-pathogenic probiotic strains may act as sources or vectors of antimicrobial resistance (AMR) or virulence factor genes, especially in culture systems where antibiotics and probiotics are co-applied [7, 10]. Although such hazards remain underexplored in aquaculture settings, their implications for both human and animal health are significant and require consideration [4, 11, 12]. Farmers may be exposed to hazards through direct handling of probiotic products, while consumers may ingest foods tainted with contaminants. Meanwhile, the crop species and non-target organisms in the environment will be exposed through contact with feed or water containing the probiotic.

In addition, ecological hazards may arise from unintended environmental impacts of probiotics: probiotics introduced into open water systems may establish as invasive species, disrupting the aquatic microbial community and potentially altering ecosystem functions such as nitrogen cycling and organic matter decomposition [13, 14]. Probiotic-related disruptions to microbial community stability may also drive reduced water quality and biodiversity, with potential long-term consequences for wild and farmed aquatic species [11].

Guidance is available for the development of safe probiotics [15, 16]; however, the absence of accepted international standards and the wide variation in national governance can lead to variable product quality, poor labelling, and inadequate safety evaluations [12]. These issues may be compounded by challenges such as genetic drift of strains and introduction of contaminants during production [12, 13]. Although not hazardous in themselves, regulatory deficiencies can mean poor quality and potentially hazardous probiotic products reach the market, posing risks to the farmer, the consumer, the cultured crop species and the wider environment.

Numerous reports have characterised certain hazards associated with probiotics, including in aquaculture [17, 18], but little research has been undertaken to prioritise potential hazards according to their perceived relative importance for product safety despite the usefulness of this knowledge for enhancing regulatory oversight in mitigation. Hazard prioritisation identifies those that matter most and it is an important step in the risk assessment process, which evaluates how likely the hazards are to cause harm and how serious the consequences may be. Such insight will support regulators to develop and implement policies that focus on addressing the most serious safety concerns so that the full promise of probiotics can be realised in the aquaculture sector.

One common approach to prioritisation is to perform a Delphi exercise, which is a structured consensus method where a panel of subject-matter experts are asked to evaluate a series of questions with the aim of achieving a converged opinion [19–21]. The approach requires panellists to complete a questionnaire over several rounds, with feedback typically provided between rounds to summarise the panel's responses, thereby enabling a reconsideration and, if necessary, adjustment of judgments in light of this collective knowledge [20, 22]. In aquaculture, the Delphi method has been used recently to prioritise fish welfare issues [23], hazards impacting milkfish aquaculture in the Philippines [24], and sustainability indicators in carrying capacity models [25]. Delphi exercises are performed in a variety of ways, with the e-Delphi approach collecting data via interactions online. This approach provides several advantages, including greater efficiency, reduced costs and the ability to gather opinions anonymously and from geographically dispersed panellists [20, 21, 26].

The aim of this present study was to evaluate the relative importance of potential hazards associated with probiotic products used in aquaculture by an e-Delphi approach, according to their implications for the safety of the farmer, consumer, cultured crop species and the wider environment, including non-crop organisms.

2 | Methods

2.1 | Recruitment of Panellists

Panellists were sought who had expertise and experience pertinent to the use of probiotic products in aquaculture. This was achieved by searching in *Scopus* using the terms 'microbiology AND probiotics AND aquaculture' to identify researchers that had authored relevant publications. Authors that had published three or more peer-reviewed articles on probiotics or microbiology in aquaculture, and who had been active in relevant research in the past 5 years, were investigated further to confirm their expertise through viewing institutional and professional profiles. The researchers meeting the eligibility criteria were contacted by email and invited to participate in the study. This email summarised the study aim, the proposed e-Delphi procedure and expected time commitment, whilst two attachments were provided: (i) a participant information sheet that outlined the study objectives, data handling and confidentiality arrangements, and voluntary participation rights statement including how to withdraw from the study, and (ii) a consent form. Although there is no universally accepted criterion for a Delphi panel size, a minimum of seven participants may provide adequate rigour and this study sought to recruit ≥ 10 panellists [27, 28]. An overview of the approach taken in this present study is provided in Figure 1.

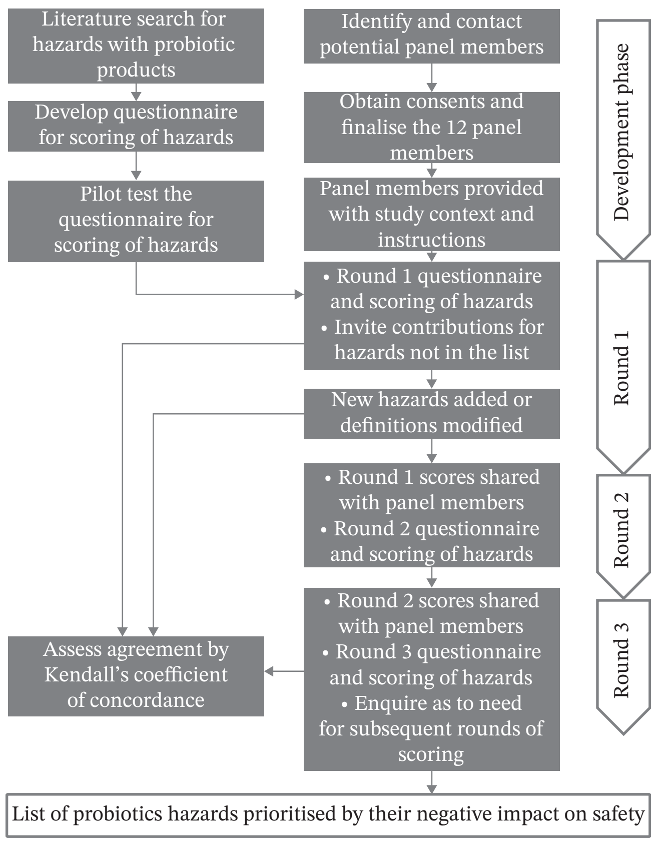

FIGURE 1 | An overview of the e-Delphi approach to evaluate the relative importance of potential hazards associated with probiotic products used in aquaculture for their impact on product safety. The key steps were to create the questionnaire used to collect evaluations, establish a panel of subject-matter experts and undertake the iterative process of evaluations.

2.2 | Literature Search for Potential Hazards

Initial literature searches were performed in *Scopus* and *Google Scholar* to identify peer-reviewed articles on characterised and theoretical hazards associated with probiotic products. Various search terms were used to initiate this sourcing of suitable articles starting with ‘probiotics AND aquaculture AND safety’, with titles and abstracts screened for relevance and reference lists interrogated for further articles. The information in the articles was used as a basis to prepare an initial list of potential hazards.

2.3 | Questionnaire Development, Piloting and Finalisation

A questionnaire was constructed as an Excel spreadsheet to contain the potential hazards, which were intrinsic probiotic product ingredients (e.g., biological factors such as microorganisms or genetic material, or chemical factors such as contaminants or toxins) or the effects such products could exert through their application (e.g., ecological factors such as disruption of existing communities). The questionnaire was completed by distributing a total of 100 points across the list of hazards to indicate their perceived relative importance according to their negative implications for the safety of each of the following receptors: (i) farmer, (ii) consumer, (iii) cultured crop species, and (iv) wider environment including non-crop organisms. The four receptors were presented in the questionnaire as four columns with 100 points attributable to each column. The total score for each of the four columns had to be 100, and a value of ≥1 had to be distributed to at least three hazards in each column. Higher values indicated greater importance for the negative impact of the hazards on product safety for that receptor.

The questionnaire was pilot-tested with two researchers locally, both of whom possessed adequate knowledge of probiotics and aquaculture. This step was used to assess the clarity of the instructions and the functionality of the questionnaire. In light of the feedback received, refinements were made to hazard descriptors and the layout of the questionnaire before it was deemed suitable for distributing to the panellists enrolled in the e-Delphi exercise.

2.4 | Data Collection by e-Delphi

2.4.1 | Round 1

Data collection commenced in June 2024. Panellists were sent the questionnaire by email with instructions for how to complete this and asked to return the file within 4 weeks. To ensure consistent interpretation, the email and the questionnaire instructions defined ‘safety’ as the potential for a hazard to negatively affect farmers (through handling or occupational exposure), consumers (through ingestion of contaminated cultured species), the cultured crop species (through disease, sub-lethal effects, or productivity losses), or the wider environment (through ecological disruption, dissemination of genetic material, or impacts on non-target organisms). Panellists were reminded that scores should reflect perceived relative importance of each hazard based on these safety dimensions. Panellists were also invited to suggest any hazards they believed to be relevant but had not been included in the list. Panellists’ identities were not revealed to each other, and the exercise would be terminated if a response rate of >80% was not attained.

Each new proposal for the hazard list was considered by the authors once all Round 1 responses had been received. Each panellist that proposed one or more new hazards received an explanation for the action taken in response, including whether the proposed hazard had been added to the questionnaire list, incorporated into an existing hazard by modifying the wording, or should be considered already within an existing hazard. New hazards linked conceptually to existing ones were inserted into the list close by to disambiguate similar issues.

2.4.2 | Round 2

For Round 2, each panellist was sent the refined questionnaire and instructions. Two points of clarification were communicated in these instructions: first, it was clarified that the hazards under consideration were only those associated with the contents of a probiotic product, not the external features such as the labels; second, it was clarified that some of the hazards in the list were known and demonstrated, but others were theoretical at present, but the scores should be the perception for the theoretical importance of these hazards if they were to be present. Panellists also received their previous scores and the means and standard deviations (SDs) calculated from the panel’s Round 1 scores. The panel scores provided each panellist the opportunity to compare their evaluations with the collective group response, which aimed to prompt reflection and possible recalibration of judgments.

2.4.3 | Round 3

Data for Round 3 were collected in the same way as Round 2, with each panellist sent the questionnaire, instructions, and the scores from the previous round. Moreover, each panellist was asked to indicate whether they expected any subsequent rounds would result in changes to their scores. This provided insight into the stability of each panellist’s opinions, the perceived adequacy of the iterative process, and whether there was a feeling amongst the panel to continue the process. The exercise would be terminated when >80% of panellists stated they would be unlikely to change their scores in subsequent rounds.

2.5 | Overall Hazard Prioritisation

The mean scores for each hazard were calculated and used to generate a ranking for the hazards for each receptor, where lower ranks indicated higher perceived importance for their negative implications for safety. An overall mean ranking for each hazard was determined by calculating the mean of the four ranks for each hazard.

2.6 | Data Analysis and Statistics

Data analyses were conducted with SPSS v29 (IBM, New York, USA). Kendall’s coefficient of concordance (W) test was applied to assess the extent of agreement amongst the panel members on the hazard rankings for each receptor, with the significance of concordance determined by chi-square test according to $\chi^2 = m(n-1)W$, where m is the number of panellists, n is the number of hazards and W is Kendall’s coefficient of concordance [29, 30].

Principal components analysis was performed to visualise the panellist scores for the hazards in Round 3 according to the four receptors, as this analysis would reveal how scores for the hazards overall were impacted by the receptor being considered.

2.7 | Ethical Approval

This study was approved by the University of Stirling General University Ethics Panel (GUEP 16815).

3 | Results

3.1 | Recruitment of Panellists and e-Delphi Response Rate

Twenty potential panellists were contacted by email and 12 agreed to participate in the e-Delphi exercise. Panellists were located in Europe (five panellists in three countries), Asia (five panellists in five countries), Africa and Australia (one panellist each). The response rate in each of the three rounds of the exercise was 100%.

3.2 | Hazards Included and Completion of the e-Delphi Exercise

Literature searches generated an initial list of 12 potential hazards associated with probiotic products (Table S1), and these were presented to the panellists in the Round 1 questionnaire. Eight panellists in Round 1 proposed additional hazards, and this led to the addition of five new hazards and modifications to two existing hazard descriptors in preparation for Round 2 (see Table S2 for the changes made). This meant that 17 hazards were scored by the panel in Rounds 2 and 3. Only four of the 12 panellists revised their assessments between Rounds 2 and 3, and all but one indicated that they were unlikely to alter their scores in any subsequent rounds, hence the exercise was terminated after three rounds.

3.3 | Most Important Hazards Impacting Farmers

After Round 3, *contamination with viable pathogenic microbial species* was the highest scored hazard for its negative impact on product safety for farmers, followed by *presence of species with transmissible virulence-related genes* and *presence of species with transmissible AMR genes* (Table 1). Meanwhile, *contamination with a dead pathogenic microbial species* was the lowest scored and thus perceived to be the least important hazard impacting product safety for farmers (Table 1). There was significant agreement amongst the panellists for the hazard rankings and Kendall's W increased from 0.2 in Round 1 to 0.45 in Round 3 (Table 2).

3.4 | Most Important Hazards Impacting Consumers

For consumers, the highest scored hazards for their negative impact on product safety was *contamination with viable pathogenic microbial species*, followed by *presence of species with transmissible AMR genes* and *presence of potentially toxic chemical contaminants* (Table 1). The lowest scoring hazard for its effect on the safety of consumers was *presence of microbial species that deplete water oxygen levels* (Table 1). There was significant agreement amongst the panellists for the hazard rankings and Kendall's W increased from 0.26 in Round 1 to 0.41 in Round 3 (Table 2).

3.5 | Most Important Hazards Impacting the Crop Species

After the Round 3 scores, *contamination with viable pathogenic microbial species* was perceived to be the most important hazard for the crop species, followed by *presence of species with transmissible AMR genes* and *presence of species with transmissible virulence-related genes* (Table 1). *Contamination with a dead pathogenic microbial species* was the hazard with the lowest score (Table 1). There was significant agreement amongst the panellists for the hazard rankings and Kendall's W increased from 0.19 in Round 1 to 0.37 in Round 3 (Table 2).

3.6 | Most Important Hazards Impacting the Environment

In addition, the hazards scored highest and thus were most important for their impact on the safety of the wider environment were *presence of species with transmissible AMR genes*, *contamination with viable pathogenic microbial species* and *presence of microbial species that deplete water oxygen levels* (Table 1). *Presence of species that negatively affect feed conversion and/or growth rate* received the lowest mean score from the panel, indicating this to be of least concern for the safety of the wider environment (Table 1). There was significant agreement amongst the panellists for the hazard rankings, and Kendall's W increased from 0.21 in Round 1 to 0.56 in Round 3 (Table 2).

3.7 | Most Important Hazards Overall

According to the mean ranking determined for each hazard for the four receptors, overall it was *contamination with viable pathogenic microbial species*, *presence of species with transmissible AMR genes* and *presence of species with transmissible virulence-related genes* that were the hazards ranked highest, and for each receptor these three hazards were within the top four ranks (Table 1). Generally, the prioritisation of the hazards was relatively similar across the receptors, with an exception being for the consumer, where *presence of microbial species that deplete water oxygen levels* and *presence of ingredients that disrupt an already healthy microbial community* scored much lower compared to the effect of these hazards on the other receptors. Furthermore, principal components analysis of panellist raw scores from Round 3 according to the four receptors showed that the points occupied a similar area of the plot, which strengthens the suggestion that the different hazards were perceived to be relatively similar for their impact on safety irrespective of the receptor being considered (Figure 3).

3.8 | Relative Importance of the Hazards

Finally, the mean raw scores from the panel after Round 3 for each of the hazards against each of the receptors provided insight into the relative weighting attached to the perceived impact of each hazard on safety (Figure 2). This more granular analysis

TABLE 1 | Mean ranks of the panel derived from their raw scores for each of the 17 hazards associated with probiotic products against each of the receptors after Round 3 of the e-Delphi exercise ($n = 12$ panellists).

Hazards	Overall		Farmer		Consumer		Crop species		Wider environment	
	Mean rank (±SD)	Actual rank	Mean rank (±SD)	Actual rank	Mean rank (±SD)	Actual rank	Mean rank (±SD)	Actual rank	Mean rank (±SD)	Actual rank
Contamination with viable pathogenic microbial species	3.0 ± 0.6	1	2.5 ± 1.5	1	3.0 ± 3.7	1	2.8 ± 3.1	1	3.8 ± 4.2	2
Presence of species with transmissible antimicrobial resistance genes (e.g., on a plasmid)	4.4 ± 0.7	2	5.3 ± 4.2	3	4.0 ± 2.4	2	4.8 ± 3.0	2	3.7 ± 1.8	1
Presence of species with transmissible virulence-related genes (e.g., on a plasmid)	5.4 ± 0.6	3	4.9 ± 2.8	2	6.2 ± 3.5	4	5.1 ± 3.2	3	5.5 ± 2.5	4
Presence of microbial species that deplete water quality such as oxygen levels	7.5 ± 3.9	4	6.9 ± 3.2	5	13.0 ± 2.5	17	6.2 ± 5.2	4	3.9 ± 2.1	3
Presence of potentially toxic chemical contaminants (e.g., heavy metals)	7.6 ± 1.8	5	7.7 ± 4.6	7	5.0 ± 4.0	3	9.3 ± 3.7	8	8.4 ± 3.2	9
Presence of ingredients that disrupt an already healthy microbial community	7.8 ± 1.9	6	5.8 ± 3.6	4	10.3 ± 3.0	10	7.2 ± 4.7	5	7.9 ± 4.2	7
Contamination with non-harmful microbial species	8.1 ± 0.9	7	7.5 ± 4.9	6	8.4 ± 4.9	7	9.2 ± 5.4	7	7.3 ± 4.1	5
Presence of antimicrobial contaminants (e.g., antibiotic, disinfectants)	8.4 ± 1.2	8	9.3 ± 3.6	8	7.3 ± 4.1	5	9.5 ± 3.8	10	7.5 ± 2.9	6
Presence of species with intrinsic antimicrobial resistance genes (i.e., on the bacterial chromosome)	8.7 ± 1.0	9	9.8 ± 1.8	9	7.6 ± 2.9	6	9.1 ± 2.8	6	8.3 ± 3.5	8
Presence of a microbe that produces antimicrobials	9.8 ± 1.0	10	11.0 ± 2.2	12	10.0 ± 2.2	8	9.6 ± 4.2	11	8.7 ± 3.9	10
Presence of potentially toxic biological contaminants (e.g., microbial toxin, enterotoxin)	10.5 ± 0.9	11	10.7 ± 4.0	11	10.2 ± 5.3	9	9.5 ± 3.9	9	11.8 ± 3.1	12
Introduction of non-native microbial species	10.8 ± 0.6	12	11.6 ± 2.4	13	10.7 ± 4.1	12	10.8 ± 3.1	12	10.2 ± 4.2	11
Ingredients ineffective for the intended purpose	11.4 ± 1.0	13	10.2 ± 5.1	10	11.9 ± 4.5	15	10.9 ± 3.8	13	12.5 ± 3.8	13
Presence of potential allergens	12.1 ± 1.1	14	12.4 ± 3.2	16	10.8 ± 4.8	13	11.7 ± 3.6	14	13.3 ± 1.6	16
Contamination with a dead non-pathogenic microbial species	12.1 ± 0.5	15	12.3 ± 4.2	15	11.5 ± 3.7	14	11.9 ± 4.0	15	12.8 ± 1.8	14
Contamination with a dead pathogenic microbial species	12.5 ± 1.3	16	13.0 ± 2.4	17	10.5 ± 2.8	11	13.2 ± 2.1	17	13.0 ± 2.0	15
Presence of species that negatively affect feed conversion and/or growth rate	12.8 ± 1.0	17	12.2 ± 3.5	14	12.7 ± 2.4	16	12.2 ± 3.5	16	14.3 ± 2.0	17

Note: Hazards are presented from top to bottom according to the overall mean ranking across the four receptors, with the most important perceived hazards at the top. Noted the slight variation in order of the hazards from Figure 2, where order was determined by mean raw scores.

TABLE 2 | Extent of agreement between panellists on their ranking of hazards for their impact on the safety of the farmer, consumer, cultured crop species and the wider environment, as assessed after each round by Kendall's coefficient of concordance (W) and chi-squared test ($n = 12$ panellists).

	Kendall's W (significance value)			
e-Delphi phase	**Farmer**	**Consumer**	**Cultured crop species**	**Wider environment**
Round 1	0.24 ($p < 0.001$)	0.26 ($p < 0.001$)	0.19 ($p < 0.01$)	0.21 ($p < 0.05$)
Round 2	0.44 ($p < 0.001$)	0.42 ($p < 0.001$)	0.33 ($p < 0.001$)	0.54 ($p < 0.001$)
Round 3	0.45 ($p < 0.001$)	0.41 ($p < 0.001$)	0.37 ($p < 0.001$)	0.56 ($p < 0.001$)

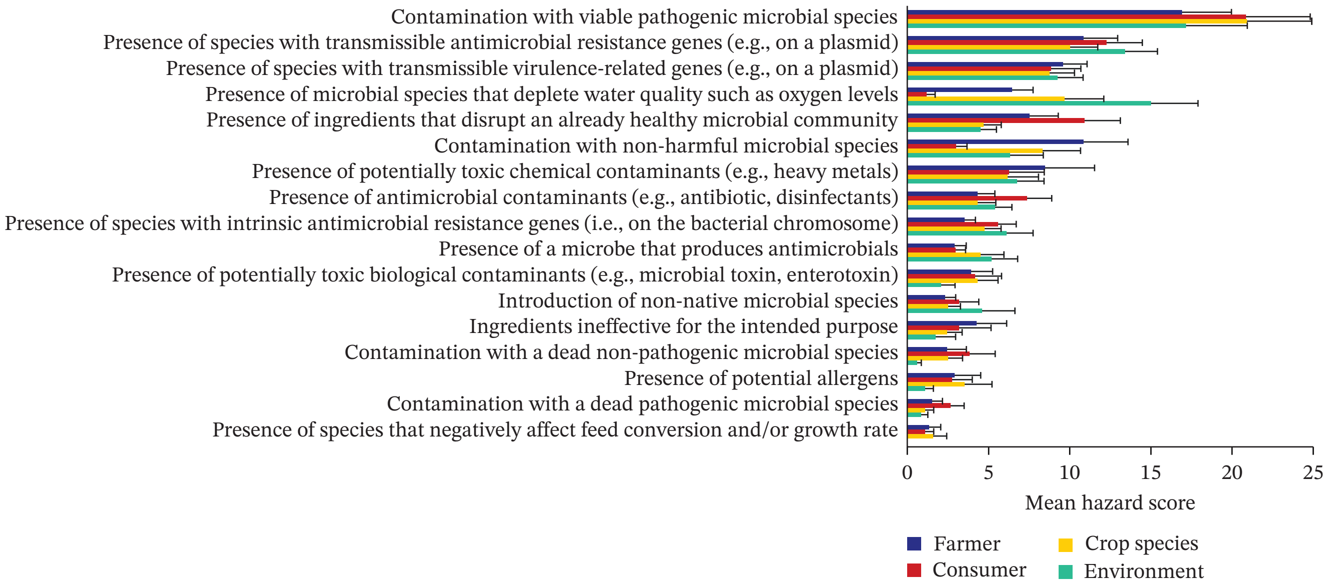

FIGURE 2 | Mean raw scores of the panel for each of 17 hazards associated with probiotic products against each of the receptors after Round 3 of the e-Delphi exercise (each bar: $n = 12$ panellists; ± 1 standard error). Hazards are presented from top to bottom according to the overall mean of means across the four receptors, with the most important perceived hazards at the top. To simplify presentation, the mean of means data can be found in Figure S1. Note the slight variation in order of the hazards from Table 1, where order was determined by mean ranks.

revealed *contamination with viable pathogenic microbial species* to be of far greater concern than the other hazards (mean raw score of 19.0), with the next four hazards having mean raw scores across the four receptors of 11.6, 9.1, 8.1 and 7.1 (Figure S1).

4 | Discussion

The aim of this present study was to evaluate the relative importance of potential hazards associated with probiotic products used in aquaculture by an e-Delphi approach. Concerns have been raised for the safety of probiotic products used in aquaculture [4–6, 12], and whilst a range of hazards have been detected, their relative importance for safe use has not been fully established. Moreover, until now, there had been relatively little attention paid to the potential impacts of hazards beyond the immediate user (i.e., farmer), but this present study included implications for animal health and welfare, system productivity, food safety and aquatic ecology.

The relative importance of 17 potential hazards was evaluated and there was relatively high agreement between panellists in the e-Delphi exercise for their perceptions of the importance of hazards associated with the intrinsic properties of products that impact safety. This consistency, and the convergence of panellist opinions over rounds, provides confidence in the findings and suggests the main safety concerns associated with probiotics were largely well recognised. Importantly, there was also high consistency in the perceived importance of the hazards for product safety irrespective of the receptor impacted, which suggests that most hazards were viewed to be broadly relevant rather than receptor specific. Overall, the consistency provides support for the establishment of risk-based approaches to probiotic safety, with the next step requiring consideration for the likelihood of a hazard to cause harm and the severity of the consequences, which may be achieved through literature analysis, further expert elicitation exercises and experimental studies to assess hazard prevalences. This would provide a platform for the development of appropriate regulatory and enforcement interventions focused on mitigating the most important hazards and risks, especially where resources are limited, as these are sorely required [12].

The panellists raised the greatest concern for the presence of potential pathogenic species of microorganisms in probiotic products. Indeed, studies have isolated a range of potential human and aquatic animal pathogens from probiotics previously, including Xu et al. [31], who isolated *Enterococcus faecium* harbouring various virulence and antibiotic resistance genes from 12 of 14 products applied as probiotics in aquaculture in China, and Noor Uddin et al. [7], who isolated *Klebsiella pneumoniae* from products used for

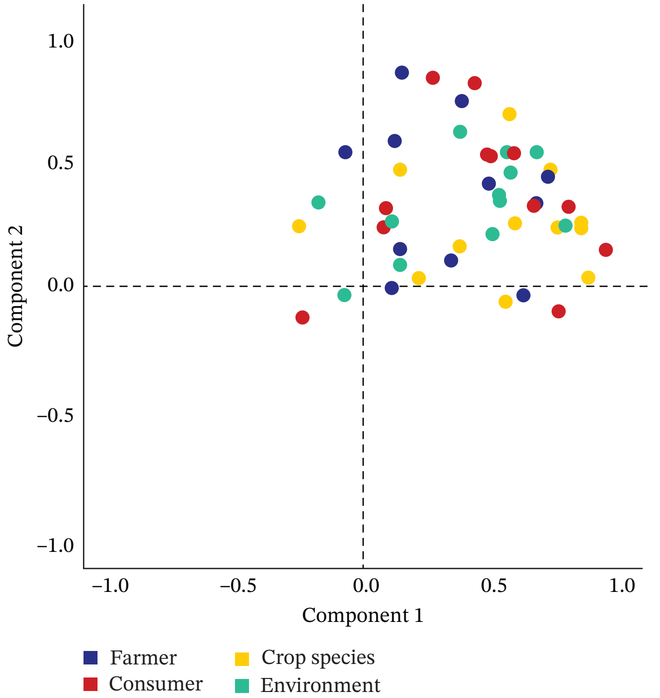

FIGURE 3 | Principal components analysis of individual panellists' scores in Round 3 according to the perceived impact of the hazards for the safety of the four receptors. This analysis suggests relative consistency in panellists' scores for the hazards across the four receptors, thus indicating the hazards were perceived to be relatively similar importance for their impact on safety irrespective of the receptor.

shrimp in Vietnam. In addition, Fu et al. [4] isolated strains of *Cronobacter sakazakii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella sonnei* and *Vibrio alginolyticus* from probiotics marketed in China for use in aquaculture, while Anokyewaa et al. [10] isolated strains of the opportunistic human pathogens *Bacillus cereus* and *B. paranthracis* that contained a range of virulence genes as detected by PCR, including enterotoxin and haemolysin genes.

The presence of mobile AMR genes also ranked very highly amongst the panellists, and this may reflect heightened current concern for this major global issue. Mobile AMR genes have also been detected in numerous previous studies of probiotics applied in aquaculture, indicating this to be more than a theoretical concern [7, 32]. Surveys of probiotic products marketed for terrestrial food animals have also identified the presence of bacterial strains harbouring transferable resistance genes on plasmids and other mobile genetic elements [4, 31, 33], and products intended for terrestrial use are often used in aquaculture too. Thus, poor-quality probiotics may represent a weakness in the global battle against AMR, with the commonly open nature of aquaculture systems leaving them well placed to receive and disseminate AMR goods, including genes and microorganisms [34, 35]. Hazardous probiotics can result from inadequate regulation or enforcement, meaning products lack rigorous safety assessments prior to production or may be manufactured in facilities that do not adhere to adequate standards [12, 13].

A closer inspection of the panellists' scores shows there were certain hazards that received scores where it is difficult to conceive how the safety of the receptor would be impacted negatively, which may suggest some misunderstanding for certain hazards in some cases. For example, some probiotic strains may inadvertently increase oxygen consumption, which could result in hypoxic conditions [8, 36], but it is difficult to see how farmer and consumer safety would be impacted by the *presence of microbial species that deplete water quality such as oxygen levels*, yet these received scores, albeit very low. Such scoring patterns are not uncommon in Delphi exercises, where lower-priority items often attract small, variable allocations due to differences in interpretation or perceived relevance [27, 28]. Moreover, there was relatively little difference between the scores used to determine the ranks of hazards perceived to be of lesser importance, which is consistent with previous studies where scoring convergence tends to be strongest for higher-priority items, while lower-rank hazards cluster with minimal separation [27, 28].

Whilst the present study yielded data that showed high consistency between panellists and convergence between rounds and benefitted from a complete response rate, several limitations deserve some consideration. Delphi exercises can be performed according to different approaches [27, 19, 20], and the e-Delphi approach employed here facilitated the creation of a diverse panel from academia, research institutes and private companies located in Europe, Asia, Africa and Australia, with anonymity mitigating the effects of dominance bias [26, 37]. The panel was created by purposive sampling that ensured panellists had suitable experience; however, this can constrain the diversity of perspectives given the members' similar professional backgrounds and training. Incorporating a broader membership can capture wider perspectives and reduce the risk that hazards are overlooked, and thus future exercises could consider expanding panel membership. Moreover, whilst the 100-point scale facilitated the acquisition of nuanced opinions, such a scheme may encourage artificial precision and impose a cognitive burden that affects the accuracy of judgments [38, 39]. Alternative scoring formats could be explored in future studies, including 5- or 7-point Likert scales, as shorter and less granular rating scales reduce respondent burden and minimise mis-responses and mapping errors [40, 41]. Furthermore, steps could be taken to mitigate positional bias, which occurs when the order in which items are presented influences how they are scored, as hazards were always provided in the same order [42]. Although consistent item order within participants supports comparability between rounds, randomising or counterbalancing item orders across participants can reduce positional bias and satisficing behaviours [19, 27]. Last, the study may also have been influenced by anchoring effects, which refers to a cognitive bias where individuals rely too heavily on initial information, such as previous mean scores from the panel, which may unintentionally influence subsequent scores [43, 44]. Anchoring can reduce the independence of judgments between rounds, potentially leading panellists to adjust scores towards the group mean rather than provide an unprompted evaluation, thereby inflating the appearance of consensus. Anchoring effects could be minimised by withholding aggregated group statistics until later rounds [19].

5 | Conclusions

In conclusion, this present study is the first to curate and prioritise a comprehensive list of hazards associated with the intrinsic properties of probiotic products used in aquaculture. The findings provide evidence to guide the focus of resources to mitigate against the most important hazards, such as enforcement of existing regulations and development of new policies. Probiotics hold huge promise for increasing productivity and sustainability in the aquaculture sector, but it is paramount that these products are safe for farmers, consumers, the cultured crop species and the wider environment.

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Disclosure

A preprint has previously been published [45].

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Any data from this study not available within the manuscript itself or the Supporting Information can be provided by contacting the corresponding author.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Supporting Information.** Table S1: List of 12 potential hazards associated with probiotic products presented to the panel in Round 1 of the e-Delphi exercise. Table S2: Changes made to the list of potential hazards associated with probiotic products from the suggestions received from the panel after Round 1 of the e-Delphi exercise. Figure S1: Mean of the four mean raw scores of the panel for each of 17 hazards associated with probiotic products against each of the receptors after Round 3 of the e-Delphi exercise.