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1 **Food safety risks in traditional fermented food from South-East Asia**

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25

26

27 **Abstract**

28
29 South-East Asia is well-known for traditionally fermented foods. However, these products are
30 generally still produced at small scale following traditional procedures. Nowadays, consumers
31 are particularly aware of the health concerns regarding food additives; the health benefits of
32 “natural” and “traditional” foods, processed with no added chemical preservatives, are
33 becoming more and more attractive. Therefore, their confidence towards safety and quality of
34 Asian fermented foods is low. Major food safety concerns are related not only to food
35 production methods, but also to how foods are processed, stored, sold and consumed. In this
36 review the main factors affecting food safety are analysed. They are not limited only to the
37 improper use of chemicals such as pesticides or antibiotics, but also to improper processing
38 and handling during storage which could provoke the accumulation of toxic compounds such
39 as mycotoxins or biogenic amines. Urgent attention is required to improve the quality of the
40 ingredients and the integration of food safety management systems for industrial growth.
41 Therefore, in the last part of this review directions to improve the food safety of fermented
42 foods are proposed.

43
44 **Keywords:** fermented foods, South-East Asia, food safety

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65 **1. Introduction**

66 In South-East Asia, the economic and demographic developments together with the migration
67 of the population to urban areas resulted in many changes in the organisation of the food
68 production system. This increased the consumer concern about food safety (Ha, Shakur, &
69 Pham Do, 2019). However, the level of concern is not only coming from an undefined
70 perception of this change but it follows also several outbreaks that occurred in the recent
71 years. Drivers of food safety unconformity have been analysed (Kendall, Kaptan, Stewart,
72 Grainger, Kuznesof, Naughton, et al., 2018). They have multiple origins among them the lack
73 of resources, the economic pressure, the structuration, the training and the will. As confirmed
74 by the achievement of the Asifood Erasmus+ project (Anal, Waché, Louzier, Roy, Mens,
75 Avallone et al. submitted), the demand of actors of the field for food safety is huge. Among
76 the various food products concerned, the case of traditional fermented food is special as these
77 foods are usually produced at small scale, very popular and considered as delicacies in Asia
78 but also, often as risky (Sarter, Ho, & To, 2014). Taking into account the classification
79 proposed by Steinkraus (Steinkraus, 1997), popular fermented food in continental South-East
80 Asia (Valyasevi & Rolle, 2002; Vu & Nguyen, 2016) are belonging to the (i) High salt/meat-
81 flavored amino acid/peptide sauce (various fish sauces or pastes like nuoc mam, prahok, pla
82 ra, soy sauces like Tuong), (ii) Lactic acid fermentations (vegetable leaves, bamboo, onions,
83 fermented meat that can be uncooked (Nem chua, nahm), fish, shrimp), (iii) alcoholic
84 fermentation (rice wine), (iv) acetic fermentation (rice vinegar), (v) alkaline fermentation of
85 soy (thua-nao). With the general economic idea of decreasing the risks and increasing the
86 scale of production, these products could be the victim of a food diversity extinction.
87 However, other economic models exist like in Europe where small-scale fermented foods are
88 considered as a way to increase typicity and bring value-added.

89 The goal of this review is to evaluate the dangers and risks to which traditional fermented
90 food are exposed in South-East Asia. Recent studies, from 2007 to 2019, concerning the
91 detection of pathogens and chemical contaminants in some of the most risky products are
92 reported. From these results, directions to improve the food safety of fermented foods in
93 Vietnam, Cambodia and Thailand will be proposed.

94

95 **2. Contamination of raw or fermented products with pathogenic bacteria**

96 In South-East Asia, and in particular in Vietnam, consumers' confidence towards fermented
97 products is low, especially because of the microbial safety risks of the raw material such as
98 meat and vegetable (Sarter, Ho, & To, 2014).

99 One of the riskiest traditional fermented meat products is an uncooked pork sausage eaten
100 after a short lactic fermentation. It is called *nem chua* in Vietnam, *nahm* in Thailand and *nem*
101 *chrouk* in Cambodia. To elaborate this product, pork is bought in the wet market or
102 supermarket and then the minced meat is mixed with herbs and spices such as garlic, guava
103 leaf, fresh chili, etc. The finish products are packed into banana leaves to provide an
104 anaerobic environment for the fermentation process and to inhibit entry of potentially
105 pathogenic microorganisms. The products are stored at room temperature for spontaneous
106 fermentation for several days.

107 The highest risk of *nem chua* is related to meat since this fermented sausage is uncooked and
108 unheated. In pork raw meat from South-East Asia, *Escherichia coli*, *Salmonella*,
109 *Campylobacter*, *Listeria monocytogenes* and *Staphylococcus aureus* are the main food-borne
110 pathogens and they are frequently found in intestinal tract and faeces of food animals (Dao &
111 Yen, 2006) (Nguyen Thi Nhung, Van, Cuong, Duong, Nhat, Hang, et al., 2018)
112 (Ananchaipattana, Hosotani, Kawasaki, Pongsawat, Md.Latiful, Isobe, et al., 2012; Carrique-
113 Mas, Bryant, Cuong, Hoang, Campbell, Hoang, et al., 2014; Dang-Xuan, Nguyen-Viet,

114 Unger, Pham-Duc, Grace, Tran-Thi, et al., 2017; T. N. M. Nguyen, Hotzel, El-Adawy, Tran,
115 Le, Tomaso, et al., 2016; Takeshi, Itoh, Hosono, Kono, Tin, Vinh, et al., 2009; Toan, Nguyen-
116 Viet, & Huong, 2013). These bacteria as well as antibiotic resistance genes can be
117 horizontally transferred to human through direct and indirect contacts with the source of
118 infection which can be animals or contaminated foods. In raw food, in the examples given in
119 Table 1, these bacteria are found with at least 10% probability of contamination. The
120 probability could rise up to 83% depending on the species, the raw food, and the origin of the
121 sample.

122 In fermented meat, the contamination is still very high in the examples presented in Table 1
123 and they even reach 100% in the neighboring regions of India (Keisam, Tuikhar, Ahmed, &
124 Jeyaram, 2019) although in one study not presenting whether fermented meat was cooked or
125 not, contaminations ranged from 0 to 19% (Ananchaipattana, et al., 2012). A study focusing
126 on *Salmonella* in the food chain and in nem chua confirmed through serovar analysis and
127 genotyping that contamination of the raw meat was usually responsible for contamination of
128 the fermented product (Le Bas, Hanh, Thành, Cuong, Quang, Binh, et al., 2008). The
129 microbial safety of *nem chua* in Vietnam has been discussed in several studies ((Phan, Pham
130 et al. 2006; Nguyen [et al.](#), 2010; 2013 ; Le, Do et al. 2012). Indeed, the concentration of *E.*
131 *coli* and *S. aureus* in raw meat for preparing *nem chua* was 10-100 and 100-1000 fold higher
132 than the National Vietnamese Standard (TCVN 7046:2002 for fresh meat), respectively.
133 Consequently, the final products *nem chua* could not meet the requirement for hygiene and
134 safety standard (TCVN 7050:2002 for unheated fermented products) (Le, Do, Le, Tran, &
135 Van, 2012; Phan, Pham, & Hoang, 2006). However, cases of illness in human beings due to
136 the consumption of *nem chua* have been rarely reported in Vietnam. Several explanations
137 can contribute to this fact, including small scale production combined with incomplete
138 epidemiological data, as well as the presence of a number of lactic acid bacteria (LAB)

139 present in the final product. These LAB and their metabolites can produce organic acid and
140 bacteriocins able to inhibit the growth of pathogenic microorganisms. The LAB of 30 samples
141 of *nem chua* were isolated and the presence of *Lactobacillus plantarum* (prevalence of
142 67.6%), *Pediococcus pentosaceus* (21.6%), *Lactobacillus brevis* (9.5%) and *Lactobacillus*
143 *faracinis* (1.3%) was reported (Tran, May, Smooker, Van, & Coloe, 2011). *L. plantarum* was
144 also reported as the most prevalent in meat and legume fermentation products (La Anh, 2015;
145 D. T. L. Nguyen, Van Hoorde, Cnockaert, De Brandt, Aerts, & Vandamme, 2013). Some
146 authors investigated thus the antibacterial properties of some *L. plantarum* strains isolated
147 from fermented food. *L. plantarum* B33 isolated from *nem chua* could inhibit food-borne
148 bacteria including *E. coli* NC31, *E. coli* K12TG1, *E. coli* 320 LCB, *S. aureus*, *S.*
149 *Typhimurium* with a zone of inhibition ranging from 4-14 mm (Lê, Hò, Trần, Chu, Lê, Lê, et
150 al., 2011). Recently, the bacteriocins produced by other LAB isolated from *nem chua* were
151 characterized (Pilasombut, Rumjuankiat, Ngamyeesoon, & Duy, 2015). It was shown that *L.*
152 *plantarum* KL-1 could produce a bacteriocin inhibiting the growth of pathogens and of some
153 LAB such as *Lactobacillus sakei*, *Leuconostoc mesenteroides* and *Enterococcus faecalis*.
154 Additionally, 47% LAB isolates from *nem chua* exhibited strong antimicrobial activity
155 against moulds from the same products and *L. plantarum* and *P. pentosaceus* showed the best
156 antifungal activities (Phong, Van, Thanh, Long, & Dung, 2016). These strains could thus be
157 useful as backslop and/or starter cultures.

158 Vegetables can also be the source of microbiological contamination (Ha et al., 2019). In
159 South-East Asia, there are different kinds of fermented vegetables including cucumbers,
160 mustard greens, young melons, cabbages, chinese cabbages, papayas, bamboo shoots, and
161 bean sprouts with Asian spider flower. They are usually produced in small-scale using mostly
162 spontaneous fermentation and sometime back-slopping fermentation. The production of
163 fermented foods and beverages through spontaneous fermentation and back-slopping

164 represents an inexpensive and reliable preservation method in less developed countries
165 (Owens, 2014). Most of the sellers of these fermented vegetables are producers and these
166 products are usually sold in the local markets in open containers. The results of two studies
167 carried out in Phnom Penh wet markets are reported in Table 1.

168 In these studies, the microbiological quality of fermented vegetables sold in Phnom Penh
169 markets was investigated, showing a correlation between microbial contamination and
170 hygienic conditions (quality and material of container, use of hands, hands with gloves, the
171 same rice paddle for all the products, open containers, etc...). The authors supposed that the
172 lack of hygienic precautions and bad cultural practices could be extended upward to the
173 production of vegetables (González García, Fernández-López, Polesel, & Trapp, 2019).

174

175 This paragraph deals with pathogen bacteria but it should be noted that another source of
176 disease has been characterized in Thai fermented fish. Indeed, liver fluke in its development
177 cycle is ingested by fishes that, if eaten without cooking, can inoculate the disease in human
178 consumers (Sriraj, Boonmars, Aukkanimart, Songsri, Sripan, Ratanasuwan, et al., 2016). It is
179 particularly dangerous as liver fluke can be a factor of development of cholangiocarcinoma,
180 especially when it is linked to high concentrations of nitrosamine (Mitacek, Brunnemann,
181 Suttajit, Martin, Limsila, Ohshima, et al., 1999). However, it can be noted that the level of
182 such cancers is not higher in North-East Thailand, the region where this fermented fish is
183 produced and consumed.

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187 **3. Toxin or toxic compounds producing microorganisms**

188

189 **3.1 Mycotoxin risks**

190 Mycotoxins are toxic secondary fungal metabolites mainly produced by five genera of
191 filamentous fungi i.e. *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium* and *Penicillium*.
192 These molds can produce different types of mycotoxins, among them, some are unique to one
193 species, but most can be produced by several fungi (Bräse et al., 2009). FAO reported that
194 25% of the world's crops are affected by mold or fungal growth with losses of around 1
195 billion metric tons of food products annually. Economic losses occur because of: 1) yield loss
196 due to diseases induced by toxigenic fungi; 2) reduced crop value resulting from mycotoxin
197 contamination; 3) losses in animal productivity from mycotoxin-related health problems; and
198 4) human health costs. Moreover, additional costs associated with mycotoxin also include the
199 cost of management at all levels (prevention, sampling, mitigation, litigation, and research
200 costs). These economic impacts affect all along the food and feed supply chains: crop
201 producers, animal producers, grain handlers and distributors, processors, consumers, and the
202 society as a whole (due to health care impacts and productivity losses). Nowadays, more than
203 400 mycotoxin metabolites have been discovered but the eight most important mycotoxins
204 (Top 8) with worldwide relevance in regard to public health are aflatoxins, ochratoxin A,
205 fumonisin B₁, zearalenone, deoxynivalenol, nivalenol, T-2 toxin and patulin (FAO, 2001).
206 Mycotoxin can contaminate various agricultural commodities and particularly cereals and
207 legumes such as wheat, barley, rye, oats, rice, maize, peanuts, alfalfa, clover, beans, peas,
208 chickpeas, lentils, lupine, mesquite, carob, soybeans, tamarind and some other cereal grains
209 that are normally used as substrates for traditional fermented food in South-East Asia. Those
210 substrates can be contaminated with mycotoxin either before harvest or under post-harvest
211 conditions (FAO, 1991), resulting in mycotoxin contamination in the finished products.
212 Mycotoxin is relatively stable to cooking and processing temperatures. Once they contaminate

213 food and feed, they cannot be removed safely. This means that once foods are contaminated,
214 human exposure is almost certain if the foods go into the market.

215 In South-East Asia, this problem is acute due to favorable humidity and temperature
216 conditions for the development of molds. It is even hypothesized that the consumption of
217 aflatoxin contaminated foods, which is recognized as a risk factor for human hepatocellular
218 carcinoma, may contribute to the high incidence of this disease in South-East Asia (Tran-
219 Dinh, Kennedy, Bui, & Carter, 2009). Several studies report a high level of contamination of
220 agricultural products and, due to the great stability of mycotoxins, these compounds remain
221 present in agriculture soils (Tran-Dinh, Kennedy, Bui, & Carter, 2009). They also remain in
222 the raw product after processing and, if the raw material is used for animal feeding, they can
223 contaminate meat products. As a result, despite a 20-year-old study showing in the north of
224 Thailand that vegetarians can be more exposed to mycotoxins (Vinitketkumnuen,
225 Chewonarin, Kongtawelert, Lertjanyarak, Peerakhom, & Wild, 1997), animal products are
226 also likely to be contaminated. For instance in Vietnam, aflatoxins and zearalenone were
227 found in all feed samples analyzed (Thieu, Ogle, & Pettersson, 2007) and then aflatoxin M1,
228 in more than half of pig urine samples collected in various slaughterhouses of Vietnam (Lee,
229 Lindahl, Nguyen-Viet, Khong, Nghia, Xuan, et al., 2017). As a result, mycotoxins can be
230 expected in fermented products in a way similar to unfermented ones (Sivamaruthi, Kesika, &
231 Chaiyasut, 2018). Although analyses of mycotoxins present in fermented products are rare,
232 some have been detected in soy fermented products like Thua-nao (Petchkongkaew,
233 Taillandier, Gasaluck, & Lebrihi, 2008) and Tuong (Vu & Nguyen, 2016). This latter soy
234 sauce is fermented by *Aspergillus orizae* which is very near, and considered as the
235 domesticated form of, *A. flavus*, the aflatoxin producer, a fact that can explain the presence of
236 aflatoxin in 4/14 brands of Tuong. A review presenting mycotoxins in world fermented

237 products has been published recently confirming that mycotoxins are often present in this
238 class of products (Sivamaruthi, Kesika, & Chaiyasut, 2018).

239

240 **3.2 Biogenic amines**

241 Biogenic amines (BAs) are low molecular weight nitrogenous compounds naturally present in
242 animals, plants, and microorganisms where they play several functions including gene
243 expression regulation, cell growth and differentiation, etc. (Suzzi and Torriani, 2015). On the
244 basis of their chemical structure, BAs are divided into aliphatic (putrescine, cadaverine,
245 spermine and spermidine), aromatic (tyramine and phenylethylamine) or heterocyclic
246 (histamine and tryptamine). Considering the number of amine groups, they are classified in
247 monoamines (tyramine and phenylethylamine), diamines (putrescine and cadaverine) or
248 polyamines (spermine and spermidine) (Park et al., 2019). The occurrence of some BAs
249 (histamine, putrescine, cadaverine, tyramine, tryptamine, 2-phenylethylamine, spermine and
250 spermidine) in fermented foods such as fish, meat, cheese, vegetables, and wines, has been
251 widely described (for a review see Spano et al., 2010). Unfortunately, the consumption of
252 foods or beverages containing high amounts of BAs is a risk for consumer health since they
253 can have toxic effects (Park et al., 2019). The BAs encountered in fermented foods are mainly
254 produced by microbial decarboxylation of amino acids (Mah et al., 2019). The main microbial
255 groups associated with BAs accumulation are several Gram negative (enterobacteria and
256 pseudomonads) and Gram positive bacteria including staphylococci, *Bacillus* spp. and lactic
257 acid bacteria (LAB). The presence of decarboxylase positive microorganisms is not the only
258 factor influencing BAs accumulation in foods, in fact, specific environmental conditions are
259 required (e.g. availability of BAs precursors, presence of proteolytic enzymes involved in the
260 release of free amino acids). Other factors involved are: raw materials characteristics in terms
261 of composition, pH, ion strength, physico-chemical parameters (NaCl, pH and ripening

262 temperature) and processing, storage and distribution conditions (Suzzi and Torriani, 2015;
263 Linares et al., 2012).

264 Several traditional fermented foods from South-East Asia including fish sauce and fermented
265 soybean foods are characterized by a high amount of BAs (for reviews see Prester, 2011,
266 Zaman et al., 2009; Park et al., 2019). The most popular fermented soybean foods are
267 produced through bacterial fermentation and the main are: Natto, Miso, Cheonggukjang,
268 Doenjang, Gochujang, Chunjang, Doubanjiang, and Douchi. The BAs composition of these
269 products is reported in Table 2 (it should be noted that for Doenjang, the very high maximal
270 concentration detected comes from one isolated study and most studies report values below
271 thresholds).

272 Fermented fish products represent a source of BAs and especially histamine, which is one of
273 the most dangerous for human health. In fact, it is the only BA with regulatory limits. The US
274 FDA set a guidance level of 50 mg/kg for histamine in the edible portion of fish (FDA, 2011),
275 and the European Commission established up to a maximum of 200 mg/kg in fresh fish and
276 400 mg/kg in fishery products treated by enzyme maturation in brine (EFSA, 2011). BA
277 content in seafood for South-Korea and China is regulated imposing maximum limits of
278 histamine content in fish, at 200 mg/kg and 200–400 mg/kg, respectively. According to EFSA
279 (2011) dried anchovies and fish sauce are the fermented foods showing the highest mean
280 content of histamine, with values of 348 mg/kg and 196-197 mg/kg, respectively. More in
281 general, fish sauce is characterized by the highest mean values for the sum of BAs (582 - 588
282 mg/kg). The histamine content of some Asian fish products is reported in Table 3.

283

284 **4. Chemical contaminations**

285

286 ***4.1 Pesticides, heavy metals***

287 In the past century, agriculture has increased productivity through mechanization,
288 fertilization, pesticides, and selective breeding. Furthermore, intensification of cash-crop
289 production and conventional agriculture with fertilizers and pesticides impair local resources
290 (soil fertility, biodiversity). Some trace elements (e.g. iron, potassium, etc...) have undergone
291 historical decrease in food in Finland, United States and United Kingdom (Mayer, 1997;
292 Ekholm et al., 2007). This decline was attributed to varietal selection based mainly on yield
293 and soil degradation due to intensive agriculture.

294 Great transitions are at work in Europe. The Ecophyto plan is implemented with the purpose
295 of progressively reducing the use of pesticides. With international trades, food products are
296 imported from numerous countries where the environmental rules do not correspond to
297 European ones. In Northern Europe, foods imported from Asia contained 111 distinct
298 pesticides and in some cases concentration exceeded the Maximum Residue Levels with leafy
299 vegetables particularly concerned (chives, thai basil) (Skretteberg, 2015). In 2010, among 245
300 plant foods from Phnom Penh markets, 15% of the long beans and 95% of the kale contained
301 noticeable levels of organophosphate and carbamate pesticides (Neufeld et al., 2010).

302 It is true that, apart from the war inheritances like agent orange which still results in daily
303 intakes of dioxins far above the WHO recommendation in some parts of Vietnam (Tuyet-
304 Hanh, Minh, Vu-Anh, Dunne, Toms, Tenkate, et al., 2015), South-East Asian cultures can be
305 potentially contaminated by chemicals and metals. Several studies have highlighted the
306 poisoning of agriculture workers (Thetkathuek & Jaidee, 2017; Thetkathuek, Suybros,
307 Daniell, Meepradit, & Jaidee, 2014) and of the environment (Harnpicharnchai, Chaiear, &
308 Charerntanyarak, 2014) but the main concern for fermented food products is related to
309 pesticides encountered in raw materials. A survey in the Red river delta showed that
310 pesticides used in agriculture were frequently detected in biota, leading to repeated analyses
311 above the acceptable daily intakes in fishes and vegetables (Hoai, Sebesvari, Minh, Viet, &

312 Renaud, 2011). Even banned organochlorine pesticides still persists in these environments.
313 Among vegetables reaching the residue limits established by the European Union, some
314 vegetables used for fermentation like chinese cabbage and some herbs are cited (Sapbamrer &
315 Hongsibsong, 2014; Wanwimolruk, Kanchanamayoon, Phopin, & Prachayasittikul, 2015)
316 while some fruits (watermelon and durian) despite the presence of chemicals, were considered
317 as safe (Wanwimolruk, Kanchanamayoon, Boonpangrak, & Prachayasittikul, 2015). The fate
318 of pesticides in food products depends on the specific physicochemical properties of the
319 compounds as well as of the conditions of preparation of food (presence of water, temperature
320 and pH etc). During fermentation, the concentration of pesticides usually decreases
321 significantly, giving rise to degradation products. In addition to non fermented products,
322 fermented products are characterized by the presence of microorganisms. The presence of
323 pesticides as well as of their degradation products tend to limit the activity of microorganisms
324 and modify the sensorial properties of the product but in the mean time, microorganisms can
325 degrade these products and help decreasing the contamination of fermented products
326 (Regueiro, López-Fernández, Rial-Otero, Cancho-Grande, & Simal-Gándara, 2015). Studies
327 on pesticides in food are still at the preliminary level and their degradation, the risk related to
328 their degradation products, the potential catalysis by microorganisms, thought of paramount
329 importance, need to be further studied.

330

331 **5. Antibiotics resistance of microbes from traditional fermented foods from South-East** 332 **Asia**

333

334 Antibiotics are either microbial secondary metabolites or the analogous compounds
335 synthesized or semi-synthesized chemically, that could inhibit the growth and survival of
336 other bacteria. These compounds are used as therapeutic agents against infectious disease in

337 humans, livestock and aquaculture. However, haphazard and extensive use of antibiotics may
338 select antibiotic resistant bacteria (Ben et al., 2019). Bacteria can develop antibiotic resistance
339 by several mechanisms via enzymatic degradation; antibiotic target modification; changing
340 the bacterial cell wall permeability and alternative pathways to escape the activity (Verraes et
341 al., 2013). Antibiotic resistance can be inherited or acquired. Inherited antibiotic resistance is
342 exhibited by all isolated of the same species while acquired antibiotic resistance occurs when
343 susceptible bacteria gain the genes encoding a resistance mechanism via mutation or the
344 transfer of genetic material from other bacteria (MacGowan & Macnaughton 2017).

345 Foodborne diseases not only affect people's well-being, but also cause hospitalization and
346 economic loss. Approximately 22.8 million cases of diarrheal illness caused by Salmonellosis
347 outbreak annually, with 37,600 deaths in South-East Asia (Van et al., 2012). Generally, food
348 contributes as an important part for transfer of antibiotic resistance in terms of antibiotic
349 residues or resistant genes from food microflora to pathogenic bacteria (Akbar & Anal, 2013).

350 It is important to monitor the prevalence of pathogenic bacteria along with antibiotic resistant
351 foodborne pathogens in food chain to improve and implement food safety (Chanseyha et al.,
352 2018). For instance, for the contamination of raw products by pathogenic bacteria as
353 discussed above, the resistance of bacteria to antibiotic is thus also an important issue. The
354 resistance of *E. coli* to antibiotic isolated from pork collected in pig farm, supermarket and
355 wet market from Hochiminh city and Tien Giang province was reported. The resistance to
356 tetracycline, sulphafurazole, ampicillin/amoxicillin, trimethoprim, chloramphenicol,
357 streptomycin, nalidixic acid, ciprofloxacin, gentamicin, colistin and ceftazidime were 100, 70,
358 55, 60, 50, 65, 30, 42.2, 73.3, 22.2 and 1.1%, respectively (N. T. Nguyen, Nguyen, Nguyen,
359 Nguyen, Nguyen, Thai, et al., 2016; Van, Chin, Chapman, Tran, & Coloe, 2008). Nguyen et
360 al. (2016a) described for the first time a strain – isolated from pork – resistant against colistin

361 (a last-resort antibiotic). Multi-resistance of *E. coli* to at least 3 different classes of antibiotics
362 was observed with rates up to 75% in pork (Van, Chin, Chapman, Tran, & Coloe, 2008).
363 *Campylobacter* and *Salmonella* spp. from pork meat showed high resistance rate (>50%)
364 against streptomycin and tetracycline in addition, with different levels of antimicrobial
365 susceptibility to ciprofloxacin, nalidixic acid, ampicillin, chloramphenicol, erythromycin and
366 streptomycin (0-50%) (T. N. M. Nguyen, et al., 2016; Vo, van Duijkeren, Gaastra, & Fluit,
367 2010). Moreover, 52.2% of *Salmonella* strains from pork, beef and chicken meat showed
368 multidrug resistance (Nguyen Thi Nhung, et al., 2018).

369 Traditional fermented foods and beverages are popular for their nutritional balance and food
370 security. In many Asian countries, techniques for fermenting cereals, vegetables and meat
371 products are well developed. Such fermented foods are highly prized for improved nutritional
372 and organoleptic quality as well as for beneficial microorganisms (Anal, 2019). Several
373 studies have indicated that along with beneficial microorganism, fermented foods can act as
374 vehicles of antibiotic resistant bacteria (see Table 4). Therefore, through the food chain
375 involving traditional fermented food, antibiotic resistant genes may be transferred to other
376 bacteria including pathogens and commensals and into the gastrointestinal tract (Abriouel et
377 al., 2017).

378 South-East Asia region is rapidly developing, and food products are exported globally. Since
379 this region is a hotspot of antibiotic resistant bacteria, there is a risk of dissemination of
380 antibiotic resistant bacteria and genes to consumers worldwide (Nhung et al., 2016). In Asia,
381 numerous fermented foods are categorized into five groups including fermented soybean; fish;
382 vegetable; bread and porridges; and alcoholic beverage. Lactic acid bacteria (LAB) are the
383 commonly involved bacteria in these products to a varying extent, having either positive or
384 negative effects (Rhee *et al.*, 2011). Some of the antibiotic resistant LAB isolated from
385 traditionally fermented foods are summarized in the Table 4.

386

387 **6. Analysis and main recommendations**

388 Despite huge differences between South-East Asian countries, concerns about risk of
389 pathogens in fermented meat product such as *nem chua*, due to the contamination in raw meat
390 material, are common (Hoang & Vu, 2017). The high level of contaminations in farms can be
391 related to hygiene (Dang-Xuan, Nguyen-Viet, Pham-Duc, Unger, Tran-Thi, Grace, et al.,
392 2019). However, several studies have highlighted an increase in the level of contaminations in
393 slaughterhouses. These contaminations were likely to come from faeces to carcasses (Dang-
394 Xuan, et al., 2019; Le Bas, et al., 2008). Cross contaminations between species have also been
395 observed as shown in Table 1: although infections by *Campylobacter* concerned mostly
396 poultry and poultry products, this species was also found in swine carcasses and retail
397 products as the meat could be contaminated with faeces at the slaughterhouse and processing
398 facilities during the evisceration process, leading to the contamination of food products (T. N.
399 M. Nguyen, et al., 2016). Taking into account that pigs are still mainly slaughtered in small
400 structures in Vietnam despite the existence of big modern structures, one axis of improvement
401 could be the improvement of hygiene conditions in those small slaughterhouses in a way
402 sustainable for small structures. These recommendations for good hygiene practices could be:
403 (1) separate gut rinsing and carcass dressing, (2) separate lairage and carcass dressing, (3) use
404 specific working surface for the carcass during slaughter, (4) cleaning and disinfection of
405 these surfaces after work, (5) give clean water to the pigs at lairage (well water, not tank
406 water), (6) clean and disinfect tanks and tools after work, (7) waste management avoiding
407 contamination (Le Bas, et al., 2008). Other steps can be causes of contaminations such as
408 handling in pork shops where management of flies and handling avoiding contact between
409 meat and worker clothes could be improved (Dang-Xuan, et al., 2019). Finally, although the
410 original load of raw pork meat is certainly the main contributor to the pathogen contamination

411 in the sausage, some additional contaminations may also result from the processing,
412 especially during the forming of the sausages into the guava and banana leaves, usually done
413 by hand. Leaves being usually considered as the source of inoculation of fermenting lactic
414 acid bacteria, a maintained spontaneous fermentation would require an improvement of the
415 leave production while another perspective would be the use of starters. Recommendation
416 could also target consumers and a Thai study recommended also to cook nam before eating
417 (Paukatong & Kunawasen, 2001), however, such a step would introduce a completely new
418 way of consumption in Vietnam.

419 For vegetables, basic hygiene could improve significantly the situation concerning pathogens.
420 Contrary to the general consumer perception, hygienic problems are not limited to wet
421 markets but are also present in supermarkets. For instance, a Thai study detected almost no
422 significant contamination differences between open markets and supermarkets however, it
423 could not take into account differences concerning fermented products as they were only
424 available in open markets at that time (Ananchaipattana, et al., 2012). It is likely that a
425 training of producers and handlers to good practices could improve greatly the hygienic
426 quality of fermented products. One particular care should be taken to cross contaminations
427 which have also been observed in vegetables as mixed vegetables are far more contaminated
428 than fermented monoproducts. Moreover, the use of hurdle technologies like biopreservation,
429 involving the use of efficient starters could also contribute to improve pathogenic control
430 through sustainable solutions (Ho, 2017).

431 The problem of mycotoxin comes mainly from conditions of mold development, which can be
432 decreased through the respect of good practice, and from the high stability of concerned
433 molecules that can stay in the environment a long time after the production by molds. One
434 concern is the price of analyses, which makes difficult any actions for basic farmers. There
435 are however also some solutions to decrease the level of contamination by using adsorbant

436 materials like bentonite clays for piglet farming (Thieu, Ogle, & Pettersson, 2008) or
437 microbial catalysts able to degrade the toxins (Petchkongkaew, Taillandier, Gasaluck, &
438 Lebrihi, 2008).

439 The fluctuations in the BAs concentration suggest the lack of standardized processes.
440 Improvements in this direction could be useful. In this context, it is essential to implement
441 control strategies and develop methods to reduce BAs content in fermented products in order
442 to face consumers demand for healthier and safe foods.

443 Food contamination by pesticides is a world-wide problem but, concerning specific
444 contaminations in the region, the agricultural practices are obviously a way to improve results
445 as, for mangosteen, 97% of the fruits for local market are exceeding the MRLs whereas no
446 problems are detected for the GAP production of fruits intended for the European Union
447 (Wanwimolruk, Kanchanamayoon, Phopin, & Prachayasittikul, 2015). Organic agriculture
448 could also be a mean to improve this point but some steps are still required to secure
449 production especially in less controlled area. Indeed, the safety of organic foods is still
450 unclear as the use of untreated irrigation or washing water or inappropriate organic fertilizers
451 (i.e manure or composts) is possible (Taban et al., 2011; Nguyen-the et al, 2016). Data related
452 to the microbial communities associated to the surface of fresh fruits and vegetables have
453 shown significant differences, quantitatively and qualitatively, between conventional and
454 organic products (Leff & Fierer, 2013; Bigot et al., 2015). Using *E. coli*, *Salmonella* and
455 *Listeria* which are the most contaminating pathogen bacteria for leafy vegetables as indicator,
456 different studies have been carried out to evaluate the contamination level of organic vs
457 conventional vegetables, notably leafy ones (Tango et al., 2014; Karp et al., 2016). Results are
458 contrasted with no real trend or a slight impact on organically-grown vegetables (Leff &
459 Fierer, 2013; Tango et al., 2014). Studies on the mycotoxin contamination of agricultural
460 products have mainly been carried out on cereals or fruits and rarely on leafy vegetables (van

461 der Walt et al., 2006; Sanzani et al., 2016). Mycotoxin contaminations in organic cereal crops
462 were variable and inconclusive but their levels were sometimes higher than those observed in
463 conventional samples (Baert et al., 2006; Magkos et al., 2006; Piqué et al., 2013). In this field,
464 more studies are needed before establishing precise recommendations.

465 In all cases, training of farmers, retailers and transformers would greatly improve the
466 situation.

467

468 **7. Conclusion**

469 Fermented foods from South-East Asia exhibit several safety risks in a similar way to raw
470 agriculture products (Figure 1). The presence of pathogens is one of the main and immediate
471 risk as it contaminates many clean samples during slaughter and handling. However, the
472 presence of chemicals, biogenic amines, mycotoxins and parasites can be a more difficult risk
473 to take into account as it can be more hidden with a long-term effect. Risks could be greatly
474 diminished by the use of good agriculture/manufacturing practice, especially concerning
475 pathogen and chemical contamination and antibiotic resistance. For mycotoxin, good
476 producing and storage practice could also help a lot and, for biogenic amines, a survey of
477 strains present during fermentation might be required as a first step before strategies to avoid
478 their development. For all food safety concerns, starters can also be used for biopreservation
479 and bioremediation purposes and thus decrease risks (Ho, Nguyen, Petchkongkaew, Nguyen,
480 Chu-Ky, Nguyen, et al., in minor revision).

481 This validates the drivers of food unsafety as cited above (Kendall, et al., 2018). Among the
482 various ways to improve safety, training of all actors of the production/transformation chain
483 may be one of the main active ones. This was the subject of the AsiFood Erasmus+ project
484 which just finished recently (Anal et al., in press).and, in the future, it would be interesting to
485 evaluate the impact of training on food safety in the different partner countries. It should also

486 be added that the level of food safety in the various ASEAN countries is very diverse as a
487 result of economic development, culture, motivation (export to world region exerting a big
488 control) etc.

489

490

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496

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894 **Table 1** Contamination of raw and fermented products in Vietnam, Thailand, Cambodia and Northeast
895 India.

Species	Samples	Origin	Place	Positive samples	Ref.
In raw products					
<i>Salmonella</i>	meat		HCMc		(Nguyen T. Nhung, Cuong, Thwaites, & Carrique-Mas, 2016)
	pork		Hanoi	25%	Toan et al. (2013)
	pork	Wet market	Hung Yen	32.8%	Takeshi et al. (2009)
	pork	Wet market	Hue	44%	Dang-Xuan et al. (2017)
	pork			11.6%	Nguyen et al. (2016)
	meat	Open markets	Bangkok /Pathum Thani	83%	(Ananchaipattana, et al., 2012)
	meat	supermarkets	Bangkok /Pathum Thani	67%	(Ananchaipattana, et al., 2012)
<i>E. coli</i>	Poultry	Factories, school, hospital canteens	Hanoi	45%	(Dao & Yen, 2006)
	Raw meat (beef, pork)			21.3%	
	Fish			6.6%	
	Veg.			18.5%	
<i>Campylobacter</i>			Vietnam	10%	Nguyen et al. (2016)
			Vietnam	15-32%	Carrique-Mas et al. (2014)

			Mekong delta	53.7%	
In fermented products					
<i>Enterococcus</i> sp.	vegetables	5 wet markets	Phnom Penh	34%	Chrun, Hosotani et al. (2017)
<i>Bacillus</i> sp. coliforms				31%	
<i>E. coli</i>				24%	
<i>E. coli</i>				10%	
<i>E. coli</i>	cucumber	22 sellers from 11 wet markets	Phnom Penh	4/11 markets	Tan Reasmey (unpublished data)
coliforms				9/11 markets	
<i>S. aureus</i>					
<i>E. coli</i>	pork and fish	Open markets	Bangkok /Pathum Thani	18%	Ananchaipattana et al. 2012
<i>Salmonella</i>				9%	
<i>Staphylococcus</i>				9%	
<i>Listeria</i>				0%	
<i>Bacillus cereus</i>	soybean	markets	North-east India	100%	Keisam et al. 2019
	bamboo			13%	
<i>Clostridium botulinum</i>	Soybean			60%	
	Bamboo			15%	
	Fish			44%	
	Milk			4%	
	pork			74%	
<i>E. coli</i>	Soybean			13%	
	milk			100%	
<i>Proteus mirabilis</i>	soybean			100%	
	pork			100%	
<i>Salmonella</i>	Nem chua			Along chain	
<i>Salmonella</i>	Nahm		Bangkok	20%	(Osiriphun, Pongpoolponsak, & Tuitemwong, 2004)

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Table 2 Minimum and maximum BAs content in some fermented soybean product. BAs concentration is expressed in mg/kg. Modified from Mah et al. (2019)

Fermented soybean product	TRP	PHE	PUT	CAD	HIS	TYR	SPD	SPM
Cheonggukjang	ND-236.4	ND-40.8	ND-121.3	ND-20.2	ND-755.4	ND-2539	ND-59.2	ND-14.7
Chunjang	13.3-31.35	ND-11.8	3.26-28.59	ND-6.6	1.85-272.55	19.78-131.27	0.24-12.8	ND-2.9
Doenjang	ND-2808.1	ND-8704	ND-4292.3	ND-3235.5	ND-2794.8	ND-6616.1	ND-8804	ND-9729.5
Doubanjiang	ND-62.43	1.43-185.61	1.15-129.17	ND-0.17	ND	ND-25.75	ND-0.18	ND-1.69
Douchi	ND-440	ND-239	ND-596	ND-191	ND-808	ND-529	ND-719	ND-242
Gochujang	ND-36.6	0.7-24.8	2.5-36.4	ND-18.1	0.6-59	2.1-126.8	ND-14.5	ND-1.8
Miso	ND-762	ND-11.76	ND-23.2	ND-201	ND-221	ND-95.3	ND-28.31	ND-216
Natto	ND-301	ND-51.5	ND-43.1	ND-42	ND-457	ND-300.2	ND-478.1	ND-80.1
Soy sauce	ND-45.8	1.5-121.6	2.5-1007.5	0.7-32.3	3.9-398.8	26.8-794.3	1.5-53.1	ND-16.1

907 TRP: tryptamine, PHE: β -phenylethylamine, PUT: putrescine, CAD: cadaverine, HIS: histamine, TYR: tyramine, SPD: spermidine, SPM:
908 spermine
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Table 3 Range of histamine concentrations found in Asian fish products

Product	Origin	HIS (mg/kg)	References
Fish sauce		45-1220	
Fish paste	Taiwan	0.1-760	Tsai et al. 2006
Shrimp paste		20-1180	
Anchovy sauces	Malaysia	63-393	Saaid et al. 2009, Zaman et al. 2010
Thai fish sauce (Som-fug)	Thailand	55.1-291	Riebroy et al. 2004
Ikan pekasam from black tilapia		18.8-71.3	
Ikan pekasam from javanese carp	Malaysia	12.7-109.1	Ezzat et al., 2015

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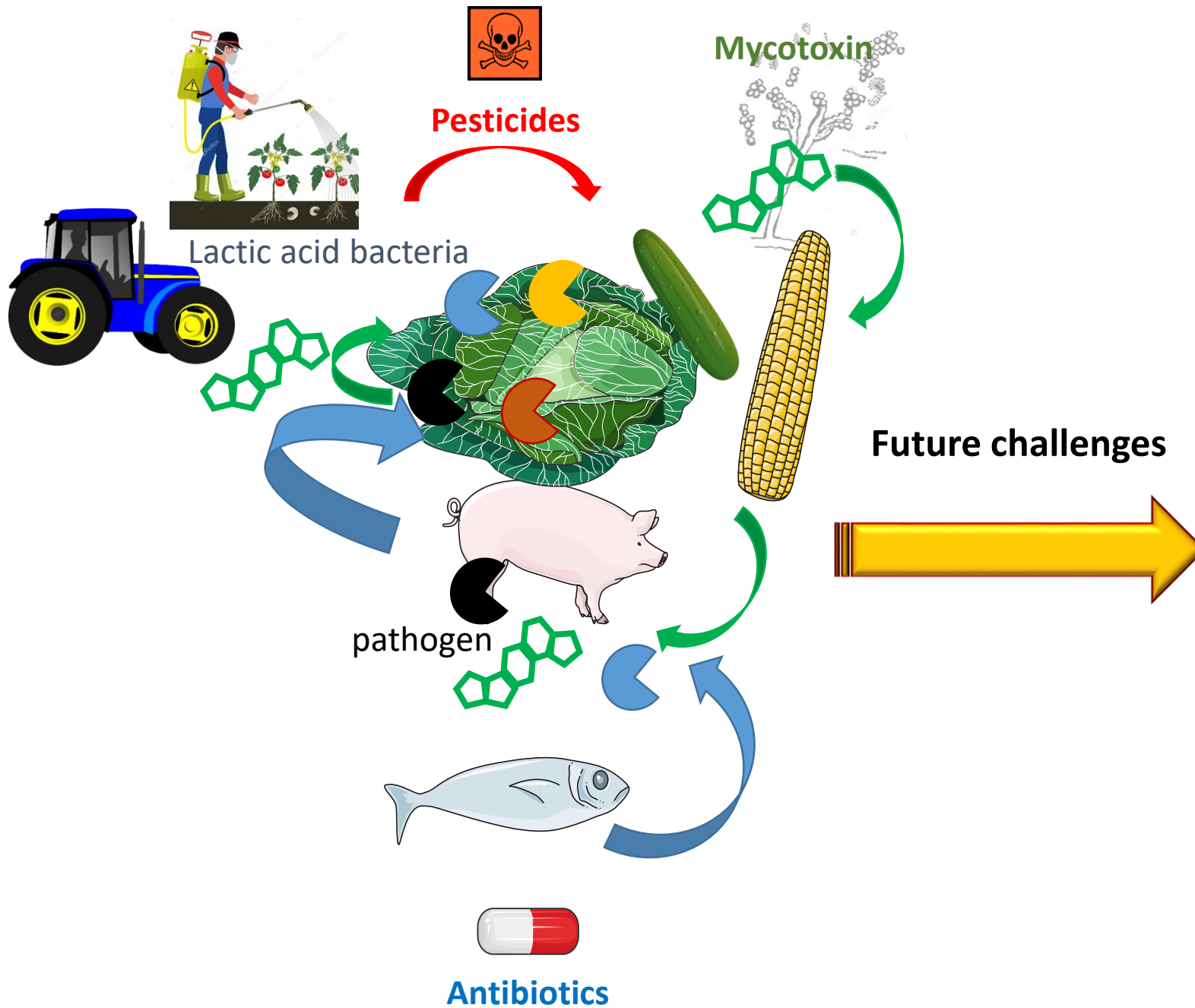
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Table 4 Antibiotic resistance of LAB isolated from Asian traditional fermented food products

Raw material	Country	Product	LAB	Phenotypic antibiotic resistance	References
Milk	China	Yogurt	<i>Lb. acidophilus, Lb. brevis, Lb. fermentum, Lb. plantarum, S. thermophilus</i>	ERY, TET	Nawaz et al. (2011)
	India	Dairy products	<i>E. casseliflavus, E. faecium, E. durans, E. lactis, Lb. fermentum, Lb. plantarum, P. pentosaceus, Ln. mesenteroides</i>	ERY, TET	Thumu and Halami (2012)
	Indonesia	Dadih	<i>Lb. fermentum, Lb. plantarum, P. acidilactici</i>	CHL, ERY	Sukmarini et al. (2014)
Meat	Indonesia	Bekasam or tempoyak	<i>Lb. fermentum, Lb. plantarum, P. acidilactici</i>	CHL, ERY	
Vegetable	China	Jiang shui	<i>Lb. plantarum</i>	TET	Nawaz et al. (2011)
		Pickle	<i>Lb. salivarius</i>	ERY, TET	
Cereal	India	Idli batter, dosa batter	<i>E. casseliflavus, E. faecium, E. durans, E. lactis, Lb. fermentum, Lb. plantarum, P. pentosaceus, Ln. mesenteroides</i>	ERY, TET	Thumu and Halai (2012)
Rice + ragi	Indonesia	Tape ketan	<i>Lb. fermentum, Lb. pantarum, P. acidilactici</i>	CHL, ERY	Sukmarini et al. (2014)
CHL, chloramphenicol; ERY, Erythromycin; TET, tetracycline					

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Figure 1°: Pre-processing



- Organic agriculture
- Standards and certifications
- Crop rotation and intercropping with nitrogen
- fixing leguminous crops
- Integrated pest management using biological controls
- Good practices
- Training

Figure 1B: Processing and post-processing

