# Epidemiology and Infection

**Carbapenem-resistant *Escherichia coli* of shrimp and salmon origin - A risk profile using the Codex framework**

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**Supplementary Material**

## Supplementary Table S1: Summary of data quality, level of concern, data gaps

| **Sections** | | **Data available (Y/N/S/L#)** | **New Data Needed to Make Risk Management Decision?** |
| --- | --- | --- | --- |
| 1. Description of the AMR food safety issue (per Codex definition) | | N/A | |
| 2. Information on the AMR organism /determinant | | Data Quality Score summary: 5.7; Level of Concern summary: 3 | |
| 2.1. Characteristics of carbapenem resistant *E. coli* (CREc) | 2.1.1. Sources and transmission routes | N | Yes  Surveillance of retail seafood in Canada, limited to nil. |
| 2.1.2. Pathogenicity, virulence, and linkages to resistance of particular strains | S | Yes  More data are needed on the genetic linkages of pathogenicity, resistance, and virulence characteristics, and its impacts on human illness. Information from whole genome sequencing (WGS) would be beneficial. |
| 2.1.3. Growth and survivability, including inactivation in foods (D-value, minimum pH for growth) of *E. coli* in seafood to fork continuum | Y | No  Guidelines for bacterial growth and survivability in seafood are published. |
| 2.1.4. Distribution, frequency and concentrations in the food chain | L | Yes  Yes, more data needed on *E. coli* and resistance prevalence/concentrations within shrimp and salmon products imported and produced in Canada to quantify the risk posed by the products to Canadians. |
| 2.2. Characteristics of carbapenem resistance in *E. coli* isolated from retail shrimp and salmon | 2.2.1. Resistance mechanisms and location of the resistance determinants | Y | No  Carbapenem resistance and their mobile genes are well characterized. |
| 2.2.2. Cross-resistance and/or co-resistance to other antimicrobial agents | Y | Yes  Even though phenotypic data exist in Canada, knowledge of the genes involved in cross-resistance/co-selection would assist decision making about interventions. Information from WGS would be beneficial |
| 2.2.3. Transferability of resistance determinants between microorganisms | Y | Yes  Antimicrobial resistance gene (ARG) transfer has been proven, however more information could be gathered about the rate of transfer. WGS would be beneficial in investigating this aspect |
| 3. Information on the antimicrobial agent(s) to which resistance is expressed - carbapenems | | Data Quality Score summary: 6.9; Level of Concern summary: 3 | |
| 3.1. Class of the antimicrobial agent(s) | | Y | No |
| 3.2. Non-human uses of carbapanems | Carbapenems are not used in aquaculture | N/A | Yes. Continued surveillance of the literature should be undertaken to identify changes in usage practices |
| 3.3 Human uses of carbapenems | 3.3.1 Spectrum of activity and indications for treatment | Y | No |
| 3.3.2 Importance of the antimicrobial agent, including consideration of critically important antimicrobial lists | Y | No |
| 3.3.3 Distribution, cost and availability | Y | No |
| 3.3.4 Availability of alternative antimicrobial agent(s) | Y | No |
| 3.3.5 Trends in the use of the antimicrobial agent(s) in humans and information on emerging diseases due to microorganism(s) resistant to the antimicrobial agent(s) or classes. | L | Specific quantitative data regarding disease manifestations related to CREc in humans are needed |
| 4. Information on the Food Commodity – salmon and shrimp | | Data Quality Score summary: 6.2; Level of Concern summary: 2.5 | |
| 4.1 Source(s) (domestic or imported), production volume, distribution and per capita consumption of foods or raw materials identified with the AMR hazard(s) of concern. | | Y | Yearly Canadian production data are available. Surveys of per capita consumption are repeated regularly (e.g. Foodbook 2.0) |
|  | 4.1.1 Characteristics of the food product(s) that may impact risk management (e.g., further processed, consumed cooked, pH, water activity, etc.) | Y | Unlike other types of animals products (e.g., poultry or beef), seafood may consumed raw (e.g., Sushi), making growing, processing and retail contamination concerning. Lacking information on different forms associated with higher probabilities of foodborne infection, or impact risk management. |
|  | 4.1.2 Description of the food production to consumption continuum (e.g., primary production, processing, storage, handling, distribution and consumption) and the risk factors that affect the microbiological safety of the food product of concern. | Y | Yes  The majority of the shrimp consumed in Canada are imported. Regulation differences concerning AMU, hygiene etc. need to be considered in any analysis of risk. There are many opportunities throughout the food production to consumption continuum for contamination to occur. Would benefit from Canadian-specific quantitative studies. |
| 5. Information on adverse public health effects | | Data Quality Score: 6.4; Level of Concern: 3 | |
| 5.1 Characteristics of the disease caused by the identified foodborne AMR microorganisms or by pathogens that have acquired resistance determinants via food | 5.1.1. Trends prevalence and nature of AMR foodborne disease in people | Y | Yes  Surveillance of CRE infections in Canada is currently undertaken by the Canadian Nosocomial Infection Surveillance Program (CNISP) and the Canadian Public Health Laboratory Network (CPHLN). Trends are increasing; Up to date Canadian passive surveillance data are available but there are data gaps with regards to the rate and prevalence of CREc, and on the burden of illness caused by CREc. However, cases attributed to food consumption are unknown. WGS analysis could provide information concerning source attribution of CREc isolates. |
| 5.1.2. Epidemiological pattern (outbreak or sporadic) regional, seasonal or ethnic differences in the incidence | S | Yes  Epidemiological patterns thus far identified are associated primarily with hospital care, where surveillance activities are concentrated. Surveillance of food animals has been undertaken by CIPARS but salmon, shrimp and seafood are not routinely included. Global dissemination of CPE is of concern. |
| 5.1.3. Susceptible population and risk factors | L | Yes  Information concerning risk factors and susceptible populations is unknown. Consideration of populations at risk, which may consume seafood raw. |
| 5.1.4. Regional, seasonal and ethnic differences in the incidence of foodborne disease due to AMR hazard(s) | S | Yes  Information concerning risk factors and susceptible populations is unknown. Consider here ethnic populations which consume seafood raw. Travel associated CRE infection has also been demonstrated. |
| 5.1.5. Consequences of AMR on the outcome of the disease Burden of illness (BOI) | N | Yes  Information examining BOI of CREc infections is limited. CRE's are associated with worse disease outcomes |
| 6. Risk management options | | Data Quality Score: 5.6; Level of Concern: 3 | |
| 6.1. Identification of risk management options to control the AMR hazard along the production to consumption continuum both in the pre-harvest and post-harvest stages | 6.1.1. Measures to reduce the risk related to the selection and dissemination of foodborne AMR microorganisms(s) | L | Farm level data is difficult to obtain from importing countries, and instigating change from importers of seafood to Canada would be difficult. Risk reduction in imported seafood products would likely target processing and retail sectors where governmental and hygienic controls (HAACP) are in place. |
| 6.1.2. Measures to minimize the contamination and cross-contamination of food by AMR microorganism(s) | S | Several studies have demonstrated the utility of HAACP programs in seafood processing plants. A qualitative description of what might be risk management options is available; quantitative data supporting the effectiveness of these measures is lacking. |
| 6.2 Effectiveness of current management practices in place based on surveillance data or other sources of information | | L | Evaluation of the effectiveness of risk management interventions is needed. WGS can be valuable in determining the impact of farming practices on AMR, virulence, and survival. It would also be useful for source attribution and control in the processing and distribution chain |
| N/A – not applicable; \*Data quality score: data were only scored as it pertains to risk, background information were not scored; #: Y-Yes, N-No, S-Some, L-Limited | | | |

**Supplementary material Table S2**

**Frequency of *E. coli* recovery and CFU concentrations in water, sediment, and seafood product at farm, processing, depot, and retail**

|  |  |  | **Sample Prevalence (%) and/or concentrations (CFU)** | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Year of study/publication** | **Level in the food-chain** | **Country** | **Water** | **Sediment** | **Shrimp** | **Salmon**  **(fish)** | **Reference** |
| 1982-1983/1985 | Retail | England (source countries not specified) | - | - | Whole - 2 (n=148)  @1.0-1.99 log10 CFU/g  Peeled – 1 (n=148)  @2.0-2.99 log10 CFU/g | - | [1] |
| NR/1994 | Retail | Brazil (Domestic source) | - | - | 33.7-40% (n=30)  Range 100->104CFU/g | - | [2] |
| NR /1994 | Retail | Bangladesh (Domestic source) | - | - | 1.2x103 (eggs) - 1.2x105 CFU/g (swimmerets) |  | [3] |
| 1994-1995/1998 | Processing unit | India | - | - | 2%  (n=1264 – raw shrimp) | - | [4] |
| 1998-2001/2005 | Ocean  Farm  Processing  Retail | Indonesia | -  <1-4.0 log10 CFU/g  -  - | -  -  -  - | <1 -3.8 log10 CFU/g  <1-4.0 log10 CFU/g  0-2.9 log10 CFU/g  <1-3.5 log10 CFU/g | -  -  - | [5] |
| 1998-1999/2003 | Processing unit | India (Domestic source) | - | - | 1.3-4.8% (n=2210) | - | [6] |
| NR/2004 | Processing unit | India | - | - | Whole prawns 50%  Headless prawns 25%  (n=18)  <20 CFU/g |  | [7] |
| NR/2005 | Processing  Landing centre  Retail | India | -  -  66 (n=12) | -  -  - | 8.6% (n=23)  16% (n=25)  15% (n=20) | -  - | [8] |
| 2001-2003/2005 | Farm | Southeast Asia (2 countries)  Central Asia (1)  Central America (1)  North America (1)  Pacific Ocean (1) | <100/100 ml or g  92.06%  100–1,000/100 ml or g  4.37%  >1,000/100 ml or g  3.57%  (n=252) | <100/100 ml or g  87.17%  100–1,000/100  ml or g  7.08  >1,000/100 ml or g  5.75%  (n=226) | <100/100 ml or g  80.57%  100–1,000/100 ml or g  12.55%  >1,000/100 ml or g  6.88%  (n=247) | - | [9] |
| 2005-2006/2008 | Freshly caught | Nigeria | -  - | -  - | 3.35x104CFU/ml  (M. vollenhovenii)  5.56x104CFU/ml  (P.atlantica) | - | [10] |
| 2009-2010/2011 | Retail | United States (Imported products from China (n=83), Chile (n=34), Thailand (n=24), Canadian and Ecuador (n=8 each), Indonesia (n=5), Mexico (n=3) Bangladesh (n=2), and Honduras, India, Norway, and Vietnam (n=1 each) | - | - | 10.5(n=38)  (Thailand (2), others (2)) | 4.8(n=63)  (Chilean salmon) | [11] |
| 2007-2008/2012 | Retail | Brazil |  |  |  | <3.0-4.6x102 MPN/g  (thermotolerant coliforms)  (n=31) | [12] |
| NR/2013 | Farm  Retail | India | - | - | 48.3% (n=60)  40% (n=40) | -  - | [13] |
| 2006-2007/2014 | Farm | Bangladesh | 8.4-14.4 (n=NRa) 408-1034 CFU/ml | 82.9-87.7 (n=NR)  4303-6056 CFU/g | 2.7-3.9% (n=NR)  189-196 CFU/g | - | [14] |
| NR/2015 | Retail, fishing harbor | India |  |  | MPN 9.5/g  (fecal coliforms) |  | [15] |
| NR/2016 | Farm  Depots | Bangladesh | 62.5 (n=16)  - | 43.7 (n=16)  - | 60.5 (n=30)  53.3% (n=45) | -  - | [16] |
| NR/2016 | Retail | India (source countries not specified) | - | - | 2%  (n=50) | - | [17] |
| NR/2016 | Retail | Germany | - | - | 31.3% (fresh)  12.5% frozen  2.59 (fresh)  log CFU/g  2.7 (frozen)  log CFU/g  (n=16) | 0-23.8% (fresh)  0-4.8 %(frozen)  <2.0-2.2(fresh)  log CFU/g  <2.0-2.3 (frozen) log CFU/g  (n=21) | [18] |
| NR/2016 | Retail | Czech Republic (source countries not specified) | - | - | - | 3.5x101-4.5x104 CFU/g  (1.6-1.7 log10 CFU/g) | [19] |
| 2006-2007/2016 | Retail | United States (National products) | - | - | - | 1.5% (combined)  (internet source, n=34)  (local source, n=32) | [20] |
| NR/2020 | Farm | Egypt | **Fishpond water inlets**  n=30  9/30 – 30%  **Tap water**  22/44 – 50%  n=44  **Outlet water**  n=26  12/26 – 46% | - | - | Fish  n=105  45/105 – 43% | [21] |

**Supplementary material Table S3**

**Antimicrobial resistance in *Escherichia coli* in wild and cultured aquatic animals and their environment**

| **Source** | **Country** | ***E. coli* prevalence**  **n/N (%)** | **Resistance phenotype (s) identified** | **Resistance genotype(s) identified** | **Associated genetic element** | **Ref.** |
| --- | --- | --- | --- | --- | --- | --- |
| Ready to eat shrimp | USA | 8/13 (62)  (Incidence) | AMP, CRO. NA, TET, TMP | - | - | [22] |
| Water and mud from culture ponds | Vietnam | Isolates grown on ATB selective media | NAL, SMX,TMP | - | - | [23] |
| Retail fish, shrimp, shellfish, processing plants, landing centers | India | Overall 73/188 (39)  Shrimp 9/68 (13) | AM, AMX, CEP, CRO, KAN, NA, PEN, STR, TET, VAN | - | Plasmids present | [24] |
| Farmed catfish | Vietnam | 11 *E. coli* isolates from pooled samples | AMP, CHL, OTC, SXT | - | - | [25] |
| Water, sediment, shrimp, fresh and saltwater fish | Malaysia, Thailand, Vietnam | Water, 39.7%  Sediment 28.8%  Fish 54.6% | CHL | - | - | [26] |
| Wild and imported shrimp | USA | 1/9 (wild)  0/13 (import) | AMP | - | - | [27] |
| Retail shellfish | Vietnam | 20 isolates selected from 50 shellfish samples | AMP, AMX/AMC, CEP, CIP, TET, CHL, ENR, GEN, KAN, NAL, NOR, SUL, TMP, NOR, STR | aadA, aphA-1, cat1, cmlA, dhfrV, sulI, , TEM, Tet(A,B) | - | [28] |
| Catfish (Ictalurus punctatus) | USA | 63 Isolates examined from enriched media | AMP, PEM, STR, BAC, RIF, CHL, SXT | dfrA12, dfrA17, aadA5 , aadA2, tetA, tetB, tetC | Integrons | [29] |
| Fish landing, processing and retail | India | 48/48 (100) | AMP, AMX, CIP, CEP, CHL, CRO NA, STR, TET, RIF | - | - | [30] |
| Shrimp, pond sediment and water | Brazil | - | AMP, CEP, GEN, **IMP**, NIT, SXT, TET | - | - | [31] |
| Retail, raw fish and RTE fish | India | 21 STEC isolates  2 non-STEC isolates from 54 raw and 27 RTE fish samples | AN, AMX, AMP, CEC, CTX, CHL, CIP, COL, SXT, ERY, GEN, KAN, LZD, NOR,OFX, PEN, PMB, STR TET, TMP | *tetA, tetB, strA, strB* | - | [32] |
| Imported seafood | Chile, Canada, China, Norway (Salmon)  Indonesia, Thailand(Shrimp) | Salmon 3/63 (4.8)  Shrimp 4/38 (10.5) | AMP, SXT, TET | - | - | [11] |
| Wholesale and retail seafood | Korea | 179/2662 (6.7%) | AMP, CAZ, CEP, CHL, CIP, GEN, KN, NA, SAM, STR, SXT/TMP, TET, TIC | Tet(A,B,D), aadA, TEM | Integron (class1 and 2)  dfrA12- aadA2, aadB | [33] |
| Retail fish (fish gut samples) | China | 218/300 (73) fish gut samples | AMP, CIP, CTF, CTX, CHL, FFC, SPC, KAN, NA, TET, SMX/TMP | Qnr, aac(6′ )-Ib-cr | Plasmids and gene co-transfer | [34] |
| River water | Portugal | Isolate grown on imipemen supplemented media | AMC, AMX, AN, ATM, CAZ, CTX, CXM, **ETP**, FEP, FOX, CIP, **IMP**, GEN, **MEM**, PIP, TIC | **blaKPC-2** | Plasmid, transposon Tn4401a | [35] |
| Retail salmon | Brazil |  |  |  |  | [12] |
| Fish farms Catfish (Clarias gariepinus) | Nigeria | 17 isolates from 90 samples | AMP, AMX, CHL, ERY, GM, NA, NOV, NIT, STR, TET | - | - | [36] |
| Retail shrimp, fishing harbor | India | MPN 9.5/g | AMP, GEN, PEN, TET, NIT |  |  | [15] |
| Retail shrimp | Vietnam | Isolates grown on cefoxatime supplemented media | AMP, CAZ, CHL, CIP, CTX, FOF, GEN, KAN, NAL, SXT/TMP, STR, TET | CTX-M, SHV, TEM | - | [37] |
| Retail shrimp | Switzerland | Salmon 3/11 (27)  Shrimp 7/11 (64) | AMP, CIP, CHL, NAL, SMX, TET, TMP | - | - | [38] |
| Retail seafood | India | Finfish 1/14 (7)  Shellfish 0/5 | CAZ, CIP, CRO, CTX, ETP, FEP, IMP, LVX, MEM, PIP/TZB | SHV, TEM, CTX-1, CTX-25, OXA-1, **NDM-5** | - | [39] |
| River water | Portugal | Isolate grown on imipemen supplemented media | All β-lactams, fluoroquinolones, and aminoglycosides (except amikacin) | blaVIM-1, blaVIM-34, blaIMP-8 | IncFIB plamid | [40] |
| Hospital food, cooked and raw fish | Iran | Raw, 1/70  Cooked 3/110 | AMP, AN, CAZ, CRO, FEP, GEN, MEZ, PMB, SMX, SXT, TET, TMP, VAN | tetA, dfrA1, aac (3)-IV, CITM | - | [41] |
| Retail seafood, venus clam | Germany | 1 *E. coli* isolate from 45 screened Enterobacteriaceae | CPM (other ARG’s derived from whole genome sequence) | *aac*A4-like, *aad*A1, *aph*(3‘)-XV, *bla*ACC-1, *bla*SHV-12, ***bla*VIM-1**, *cat*B2, *dfr*A14-  like, *mph*(A), *qnr*S1, *str*A-like, *str*B-like, *sul*1, *sul*2 | IncY plasmid | [42] |
| Retail and wholesale fish |  | 9/136 (*East1, EPEC, EAEC)* | AMC, AMP, CEP, FOX, NAL, TET | - | - | [43] |
| Retail fish, shrimp and seafood | India | 19 seafood samples, Enterobacteriaceae  ESBL+(169/215) | CAZ, CTX, CPD, IPM, ETP, FOX, MRP, CIP, ATM, AMC, TZP | blaCTX blaSHV blaTEM **blaNDM** | - | [44] |
| Wild and cultured fish stools | Brazil | STEC and EPEC screening of fish samples in BHI | AMP, CFL, TET |  |  | [45] |
| Effluent from shrimp farms | Vietnam | 1 Isolate grown on ATB selective media | - | sul1, sul2, qnrA, ermB | Plasmids? | [46] |
| Retail seafood, shrimp and shellfish | Germany (several sources some unknown?) | - | ESBL+ | blaSHV, þblaACC, blaCTX-M, blaTEM, blaCMY-2 | - | [47] |
| Retail fish | Cambodia | ESBL+ *E. coli*; 32 (53%) of 60  Fish | AMG, APL, COL, CPM, ESBL+, FLQ, MAC, SXT, TET | AMG (aad, aac, aph, strA/B), APL (cat, floR, cmlA), COL (mcr), CPM (*bla*OXA-181), ESBL (blaCTX-M, blaCMY-2)FQL (qnrSq, *aac(6')Ib-cr*, *oqxA*), MAC(erm,mph mef, Inu), SXT (SUl, dfr), TET(A,B,M) | - | [48] |
| Farmed fish  Inlet;oulet water  Tap water | Egypt | 45/105 (43%)  9/30 (30%); 12/26 (46%)  22/44 (50%) | CAZ, CRO, CTX, ETP, FOX, IPM, MEM | *bla*KPC, *bla*OXA-48, and *bla*NDM  *bla*CTX-M-15, *bla*SHV, *bla*OXA-1, *bla*TEM, and *bla*PER-1 | plasmids | [21] |

**AMC** Amoxicillin-clavulanic acid, **AMG** Aminoglycosides, **AN** Amikacin, **AMP** Ampicillin, **AMX** Amoxicillin, **APL** Amphenicol, **AZM** Azithromycin, **ATM** Aztreonam, **BAC** Bacitracin, **CAZ** Ceftazidime, **CPM** carbapenem, **CXM** Cefuroxime, **CTX** Cefotaxime, **CEC** cefaclor, **CEP** Cephalothin, **FEP** Cefepime, **CPD** Cefpodoxime, **CHL** Chloramphenicol, **CIP** Ciprofloxacin, **CLA** Clavulanic acid; **CLI** Clindamycin, **COL** Colistin, **CRO** Ceftriaxone, **ETP** Ertapenem, **ERY** Erythromycin, **FIS** Sulfisoxazole, **FLQ** Fluoroquinolones, **FFC** Florfenicol, **FOF** Fosmycins, **FOX** Cefoxitin, **GEN** Gentamicin, **IMP** Imipenem, **KAN** Kanamycin, **LVX** levofloxacin, **LZD** linezolid, **MAC** Macrolides, **MEM** Meropenem, **MEM** Meropenem, **MEZ** Mezlocillin, **NAL** Nalidixic acid, **NIT** Nitrofurantoin, **NOR** norfloxacin, **NOV** Novobiocin, **OFX** ofloxacin, **PEN** Penicillins, **PIP** Piperacillin, **PMB** polymyxin B, **OTC** Oxytetracycline, **RIF** Rifampicin, **SMX** Sulfamethoxazole, **SPC** Spectinomycin, **SAM** Ampicillin/sulbactam, **SSS** Sulfisoxazole, **STR** Streptomycin, **SXT** Trimethoprim-sulfamethoxazole, **TZB** Tazobactam, **TBC** total bacterial counts, **TEL** Telithromycin, **TET** Tetracycline, **TIC** Ticarcillin.**TIO** Ceftiofur, **TMP** Trimethoprim, **VAN** Vancomycin

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