

Detection of antimicrobial residues and foodborne pathogens in shrimps sold at traditional markets and supermarkets in southern Vietnam

Nguyen Thi Phuong Yen^a, Nguyen Thi Nhung^a, Nguyen Thi Bich Van^a, Nguyen Van Cuong^a, Le Tran Tien Chau^a, Huynh Ngoc Trinh^a, Chu Van Tuat^b, Nguyen Phu Huong Lan^c, James Campbell^{a,d}, Stephen Baker^{a,d}, Guy Thwaites^{a,d}, Juan Carrique-Mas^{a,d}

^a Oxford University Clinical Research Unit Vietnam (OUCRU); ^b National Centre for Veterinary Hygiene Inspection No I - Department of Animal Health – Vietnam; ^c Hospital of Tropical Diseases, HCMC; ^d Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine, Oxford University, United Kingdom
Contact email: yennp@oucru.org

Introduction

- In Vietnam, a large amount of antimicrobials are used in aquaculture production
- Most shrimp production in Vietnam (683,000 metric tons in 2017) is targeted at the export market
- Lack of data on foodborne hazards (antimicrobial residues, non-typhoidal *Salmonella* and *Vibrio* spp.) in shrimp products purchased locally



Aims and Objectives

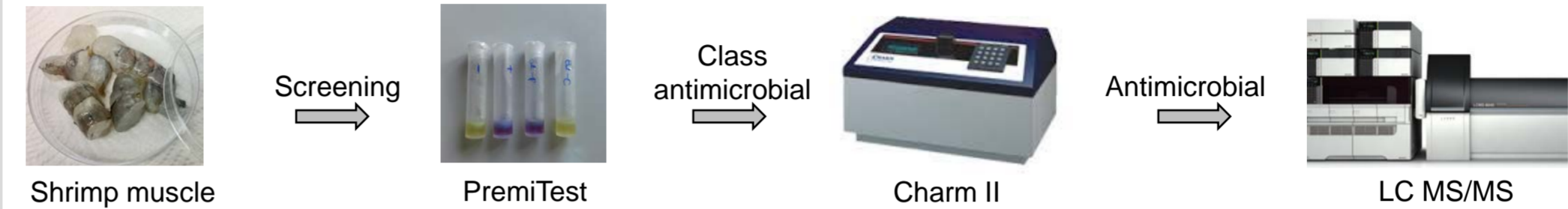
- To estimate the prevalence of antimicrobial residues in shrimp
- To investigate the prevalence of non-typhoidal *Salmonella* (NTS), *Vibrio* spp. and their antimicrobial susceptibility profiles

Materials and Methods

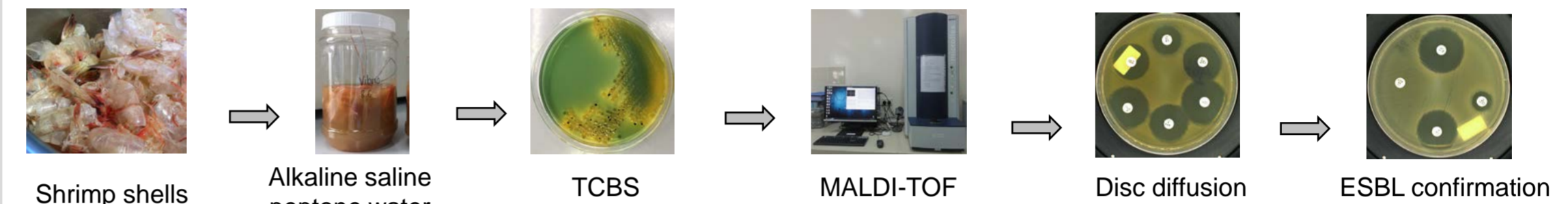
Identify shrimp



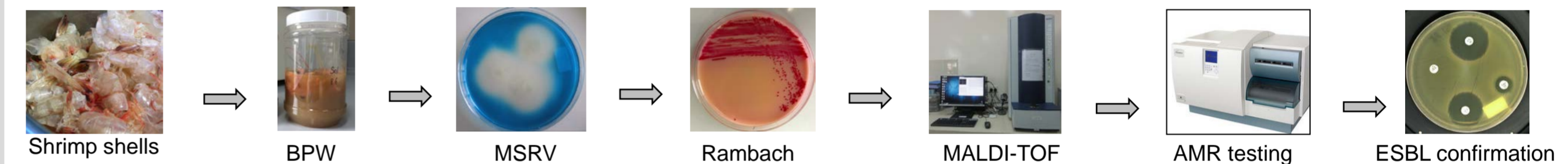
Screening and detection of antimicrobial residues



Isolation of *Vibrio* spp.



Isolation of NTS



Results

Table 1. Description of shrimp samples, prevalence of residues, NTS and *Vibrio* spp. in 40 shrimp batches

| No. samples | No. (%) positive | | | | | | | | |
|----------------------------|------------------|------|-----------------------|----------------|------------------|--------------------|------|------|---------------|
| | Premi-Test | NTS | <i>Vibrio</i> species | | | | | | V. vulnificus |
| | | | V. parahaemolyticus | V. navarrensis | V. alginolyticus | V. cholerae non-O1 | | | |
| Type of retail site | | | | | | | | | |
| Supermarket | 10 | 50.0 | 60.0 | 90.0 | 70.0 | 40 | 60.0 | 20.0 | 0 |
| Traditional market | 30 | 13.3 | 80.0 | 86.7 | 56.7 | 56.7 | 30.0 | 23.3 | 13.3 |
| Shrimp species | | | | | | | | | |
| White leg shrimp | 30 | 26.7 | 73.3 | 86.7 | 50.0 | 53.3 | 40.0 | 23.3 | 13.3 |
| Giant tiger shrimp | 5 | 20.0 | 60.0 | 80.0 | 100 | 40.0 | 40.0 | 20.0 | 0 |
| Other species | 5 | 0 | 100 | 100 | 80 | 60 | 20 | 20 | 0 |
| Condition | | | | | | | | | |
| Alive | 17 | 17.6 | 70.6 | 94.1 | 47.1 | 64.7 | 23.5 | 29.4 | 23.5 |
| Dead | 23 | 26.1 | 78.3 | 82.6 | 69.6 | 43.5 | 47.8 | 17.4 | 0 |
| Price (per kg) | | | | | | | | | |
| ≤170k VND* | 22 | 27.3 | 63.6 | 81.8 | 63.6 | 54.5 | 40.9 | 22.7 | 4.5 |
| >170k VND | 18 | 16.7 | 88.9 | 94.4 | 55.6 | 50 | 33.3 | 22.2 | 16.7 |
| Total | 40 | 22.5 | 75.0 | 87.5 | 60.0 | 52.5 | 37.5 | 22.5 | 10.0 |

*VND=Vietnam Dong (1 USD=23kVND)

Table 3. Antimicrobial susceptibility of *Vibrio* spp. isolates

| Class and antimicrobial | No. isolates and percent resistant (%) | | | | | | | Total (n=133) |
|----------------------------------|--|-----------------------|-------------------------|---------------------------|---------------------|--------------------|---|---------------|
| | V. parahaemolyticus (n=64) | V. navarrensis (n=23) | V. alginolyticus (n=21) | V. cholerae non-O1 (n=15) | V. vulnificus (n=6) | V. fluvialis (n=4) | | |
| Cephalosporins (3rd gen.) | | | | | | | | |
| Cefotaxime | 31.3 | 8.7 | - | - | - | - | - | 16.5 |
| Ceftazidime | 15.6 | - | - | 6.7 | - | - | - | 8.3 |
| ESBL | 28.1 | - | - | - | - | - | - | 13.5 |
| Quinolones | | | | | | | | |
| Ciprofloxacin | 10.9 | - | - | - | - | - | - | 5.3 |
| Ofloxacin | 9.4 | - | - | - | - | - | - | 4.5 |
| Aminoglycosides | | | | | | | | |
| Amikacin | 1.6 | - | - | - | - | - | - | 0.8 |
| Gentamicin | 1.6 | - | - | - | - | - | - | 0.8 |
| Penems | | | | | | | | |
| Imipenem | 3.1 | 4.3 | - | - | - | - | - | 2.3 |
| Penicillins | | | | | | | | |
| Ampicillin | 100 | 52.2 | 100 | 53.3 | 33.3 | 75 | - | 82.7 |
| Amoxicillin-clavulanic acid | 9.4 | 17.4 | - | 33.3 | - | - | - | 11.3 |
| MDR | 21.9 | | | | | | | 11.3 |

- Vibrio* spp.: high susceptibility to antimicrobials, except for ampicillin (82.7%)
- V. Parahaemolyticus*: high resistance toward the third generation cephalosporins (cefotaxime 31.3% and ceftazidime 15.6%; ESBLs 28.1%)

Discussion and Conclusions

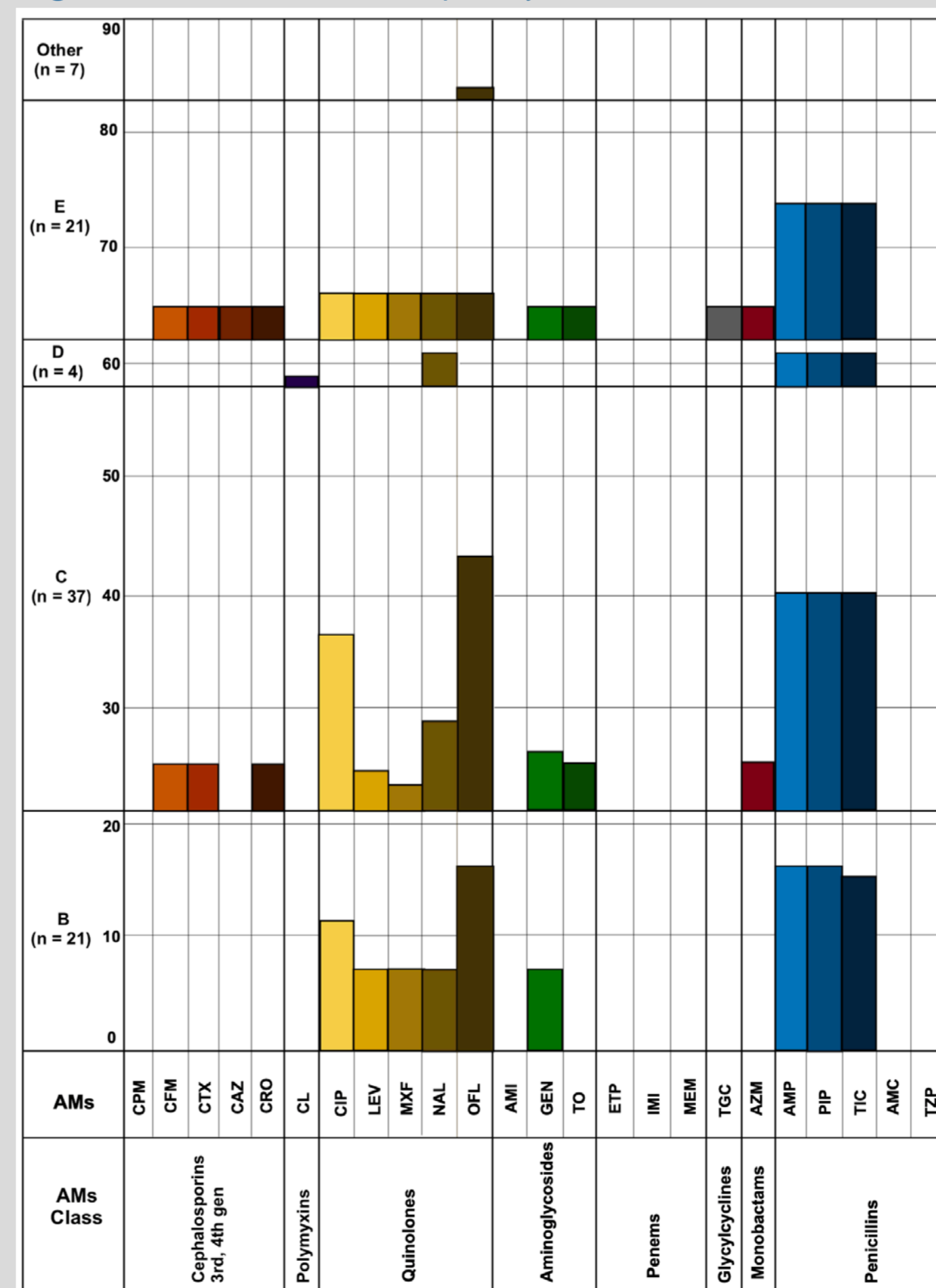
- 22.5%, 75% and 100% of samples contaminated with residual antimicrobial, NTS and *Vibrio* spp.
- Appropriate withdrawal periods should be strictly enforced after the administration of antimicrobials to ensure the safety of aquaculture food for human consumption
- We recommend restricting inappropriate antimicrobial usage on shrimp farms, and establish monitoring of antimicrobial residues and food-borne pathogens in shrimp products at retail sites

Table 2. Results of antimicrobial residue testing by Charm II and LC-MS of 9 shrimp samples that tested positive by PremiTest

| Sample | Description | Charm II (antimicrobial class) | UHPLC-MS/MS (Antimicrobial identified) | Concentration of antimicrobial active ingredient (µg/kg) | MRL (ug/kg) VN regulation |
|--------|------------------------|--------------------------------|--|--|---------------------------|
| 1 | White leg shrimp, dead | Tetracyclines | Tetracycline* | 590.7 | 100 |
| | | Sulfonamide | Sulfamethoxazole* | 157.6 | 100 |
| | | - | Flumequine** | 38.5 | 200 |
| 2 | White leg shrimp, dead | Tetracyclines | Oxytetracycline* | 122.2 | 100 |
| | | - | Ciprofloxacin** | 30 | Prohibited |
| 3 | White leg shrimp, dead | Macrolide | ND | - | - |
| | | - | Flumequine** | 41.2 | 200 |
| 4 | White leg shrimp, dead | Tetracyclines | Oxytetracycline* | 43.7 | 100 |

** Critically important AM; * Highly important AM; ND: Not detected; MRL: Maximum residue level

Figure 1. Antimicrobial susceptibility of NTS isolates



- NTS: high susceptibility to penems, polymyxins and cephalosporins (3rd and 4th gen.). ESBLs (7.8%)
- NTS: high resistance toward the quinolones, and penicillins

Antimicrobials:

CPM: Cefepime
CFM: Cefixime
CTX: Cefotaxime
CAZ: Ceftazidime
CRO: Ceftriazone
CL: Colistin
CIP: Ciprofloxacin
LEV: Levofloxacin
MXF: Moxifloxacin
NAL: Nalidixic acid
OFL: Ofloxacin
AMI: Amikacin
GEN: Gentamicin
TO: Tobramycin
ETP: Ertapenem
IMI: Imipenem
MEM: Meropenem
TGC: Tigecycline
AZM: Aztreonam
AMP: Ampicillin
PIP: Piperacillin
TIC: Ticarcillin
AMC: Amoxicillin/Clavulanic Acid
TZP: Piperacillin/Tazobactam

Salmonella serovar: (from most common serovars to less common serovars)
Braenderup (C, n=13), Anatum (D, 11), Saintpaul (B, 9), Rissen (C, 9), Derby (B, 4), Enteritidis (D, 4), Give (E, 4), Paratyphi B (B, 3), Bovismorbificans (C, 3), Infantis (C, 3), Kentucky, Litchfield (C, 3), Senftenberg (E, 3), Poona (other, 3), Typhimurium (B, 2), Ohio (C, 2), Weltevreden (E, 2); other- Kedougou (other, 2), Urbana (other, 2), Agona (B), Stanley (B), Unknown (B), Albany (C), Bareilly (C), Potsdam (C), London (E)