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Research Note

Antimicrobial Resistance of *Escherichia coli,* Enterococci, *Pseudomonas aeruginosa,* and *Staphylococcus aureus* from Raw Fish and Seafood Imported into Switzerland

RENATE BOSS,[1]* GUDRUN OVERESCH,[2] AND ANDREAS BAUMGARTNER[1]

[1]*Federal Food Safety and Veterinary Office, Schwarzenburgstrasse 155, 3003 Berne, Switzerland; and* [2]*Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Berne, Länggassstrasse 122, 3001 Berne, Switzerland*

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ABSTRACT

A total of 44 samples of salmon, pangasius (shark catfish), shrimps, and oysters were tested for the presence of *Escherichia coli,* enterococci, *Pseudomonas aeruginosa,* and *Staphylococcus aureus,* which are indicator organisms commonly used in programs to monitor antibiotic resistance. The isolated bacterial strains, confirmed by matrix-assisted laser desorption ionization time-of-flight mass spectroscopy, were tested against a panel of 29 antimicrobial agents to obtain MICs. Across the four sample types, *Enterococcus faecalis* (59%) was most common, followed by *E. coli* (55%), *P. aeruginosa* (27%), and *S. aureus* (9%). All bacterial species were resistant to some antibiotics. The highest rates of resistance were in *E. faecalis* to tetracycline (16%), in *E. coli* to ciprofloxacin (22%), and in *S. aureus* to penicillin (56%). Antibiotic resistance was found among all sample types, but salmon and oysters were less burdened than were shrimps and pangasius. Multidrug-resistant (MDR) strains were exclusively found in shrimps and pangasius: 17% of pangasius samples (MDR *E. coli* and *S. aureus*) and 64% of shrimps (MDR *E. coli, E. faecalis,* and *S. aureus*). Two of these MDR *E. coli* isolates from shrimps (one from an organic sample) were resistant to seven antimicrobial agents. Based on these findings, *E. coli* in pangasius, shrimps, and oysters, *E. faecalis* in pangasius, shrimps, and salmon, and *P. aeruginosa* in pangasius and shrimps are potential candidates for programs monitoring antimicrobial resistance. Enrichment methods for the detection of MDR bacteria of special public health concern, such as methicillin-resistant *S. aureus* and *E. coli* producing extended-spectrum β-lactamases and carbapenemases, should be implemented.

Key words: Antimicrobial resistance; Broth microdilution; Monitoring; Seafood; Switzerland

About 90% of aquaculture production occurs in developing countries *(9, 19, 24)* with poor standards for hygiene and incomplete regulations on antibiotic usage *(17, 24),* leading to contamination of sewage with antibiotics *(13, 22)*. Aquaculture businesses are often located in coastal areas or estuaries where levels of water pollution from human and animal feces are high *(3)*. Integrated agriculture-aquaculture farming systems often recycle human and livestock waste *(12)*. Massive amounts of antimicrobial agents are required to control diseases, minimize economic loss, hasten production *(17),* and satisfy the world's increasing demand for fish and seafood, which can no longer be provided by wild fisheries *(1)*. Important drugs that should be reserved for therapy of humans *(27),* particularly cephalosporins and quinolones, are sometimes applied by farmers with little understanding of the prudent use of antibiotics *(17)*. In such systems, antibiotic resistance genes are omnipresent and easily transferred to the bacterial flora of farm animals used for human consumption *(12)*. Although these findings particularly concern farms producing food for domestic markets rather than export, an eye should be kept on these types of products. Foods of animal origin, especially meat, serve as vectors for the spread of resistant bacteria, such as extended-spectrum β-lactamase (ESBL)–producing *Escherichia coli* strains *(14)* that are often detected in broiler meat. The role of foods in the transfer of antimicrobial resistance genes requires more intensively study *(4, 5, 26)*.

For risk-based management actions, solid and sufficient data on resistant bacteria are needed. Such data can be obtained by monitoring antibiotic resistances in indicator and zoonotic bacteria. Fish and seafood in Switzerland have not been monitored for antimicrobial resistance. However, a high prevalence of *E. coli* resistant to various antibiotics (up to 12) has been found in fish and seafood in Korea *(20)* and Vietnam *(25),* which suggests that these products may act as a reservoir for multidrug-resistant (MDR) bacteria and thus should be monitored. Some *Staphylococcus aureus* isolates from ready-to-eat fish products were resistant to various antibiotics (including methicillin) *(2, 11, 21)*. Fish and seafood are promoted as well-balanced and healthy sources of nutrition and have become more popular, as indicated by increasing levels of consumption *(9)*. Aquaculture is the

* Author for correspondence. Tel: +41 58 463 30 33; Fax: +41 58 463 85 70; E-mail: renate.boss@blv.admin.ch.

world's fastest growing sector for food of animal origin, and about half of all fish and seafood products now originate from farming operations *(9)*. The world's production of food fish expanded almost 12 times in the last 30 years. In Switzerland, consumption of fish and crustaceans is rising, and >97% of these products are imported *(18)*.

For these reasons, the conditions for a possible antimicrobial resistance monitoring program for imported fish and seafood products in Switzerland were evaluated, using standardized methods to allow the comparison of results in both a national and international context *(6)*. The target bacteria should be those that easily transmit resistance genes and are relevant to human medicine. The known indicator bacteria *E. coli,* enterococci, *Pseudomonas aeruginosa,* and *S. aureus* were selected for analysis based on their sufficiently high prevalence and demonstrated resistance to antimicrobial agents of public health significance.

MATERIALS AND METHODS

Samples. A total of 44 samples of salmon ($n = 11$), pangasius (shark catfish; $n = 12$), shrimp ($n = 11$), and oysters ($n = 10$) produced via aquaculture were purchased at retail shops between August and October 2014 in Berne, Switzerland. All pangasius samples originated in Vietnam and were fresh, frozen, or thawed raw filets. If declared, the species of all samples was *Pangasius hypophthalmus,* with the exception of one dried frozen sample of *Pangasius krempfi*. Salmon samples originated from aquaculture in the northern Atlantic (Scotland, Denmark, or Norway) and were fresh or frozen raw filets. Oysters originated either from France or Scotland and were purchased either loose or packed under a controlled atmosphere. Shrimp originated from Bangladesh, Vietnam, Thailand, Indonesia, or Ecuador, were bought as raw products, either frozen or thawed, and, if declared, belonged to the species *Penaeus monodon* or *Litopenaeus vannamei*.

Sample processing. A 25-g sample aliquot was homogenized with 225 ml of sterile NaCl–peptone water containing 1 g/liter pancreatic digested peptone from casein (Sigma-Aldrich, Buchs, Switzerland) in a filter stomacher bag attached to a Stomacher 400 blender (Seward Limited, Worthington, UK). From this mixture, 100 μl was directly spread on selective nutrient agar plates (see below). When no growth was obtained with the direct plating approach, 10 μl of the enriched mixture, incubated for 24 h at 37°C, was spread on the agar plates.

Bacterial species tested. For isolation of *E. coli,* tryptone bile X-glucuronide agar (Sigma-Aldrich) was used. Plates were incubated at 30°C for about 6 h and then at 44°C for 18 to 24 h. Large blue-green colonies were considered presumptive *E. coli*. For isolation of *Enterococcus faecalis* and *Enterococcus faecium*, m-*Enterococcus* agar (BD, Allschwil, Switzerland) was used. Plates were incubated at 37°C, and results were read after 24 and 48 h. Presumptive *Enterococcus* colonies (small, light pink to red) were subcultured on bile esculin agar (Sigma-Aldrich) to evaluate the esculin dehydration of *E. faecalis* and *E. faecium* after overnight incubation at 37°C. *P. aeruginosa* was isolated on cetrimide sodium nalidixate agar (Oxoid, Pratteln, Switzerland) supplemented with Pseudomonas CN selective supplement (Oxoid). The plates were incubated at 30°C for 18 h, and presumptive *P. aeruginosa* characteristically appeared as large yellowish to brown colonies, often fluorescent under UV light. For further confirmation, oxidative degradation of glucose and the presence of oxidase were analyzed with the Hugh-Leifson test using OF basal medium (Merck, Zug, Switzerland) prepared with 10% D(+)-glucose (Merck) and oxidase strips (Sigma-Aldrich), respectively. β-Hemolysis on sheep blood agar (BA; bioMérieux, Geneva, Switzerland) was often seen with *P. aeruginosa* colonies. *S. aureus* was isolated on CHROMagar Staph. aureus (CHROMagar, Paris, France), which was incubated at 37°C for 24 h. Mauve colonies were considered presumptive *S. aureus* and further confirmed by the presence of catalase and a negative potassium hydroxide test. For isolation of *Aeromonas hydrophila,* rainbow agar Shigella/Aeromonas (Biolog, Hayward, CA) culture plates were incubated for 20 to 24 h at 37°C. Presumptive strains appeared as orange to red colonies that were positive for potassium hydroxide, the presence of oxidase, and fermentative degradation of glucose, as described for *P. aeruginosa*.

One to three presumptive isolates per target organism and sample were subcultured on BA, and an aliquot was stored at −70°C until further examination.

Confirmation by mass spectroscopy. The identity of a total of 242 bacterial isolates was confirmed by matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-TOF MS) using the direct transfer protocol recommended by the manufacturer (Biotyper 3.0, Bruker Daltonics, Bremen, Germany). Material from a single colony of a fresh overnight BA culture was removed with a toothpick, smeared on a steel plate, and overlaid with 1 μl of α-cyano-4-hydroxycinnamic acid matrix solution. After air drying, measurements were made using standard settings in the Flex Control software. Analysis of spectra with the Biotyper 3.0 included a comparison against the internal commercial database and the institute's database. Species level results were accepted at a score of >1.999.

Antimicrobial susceptibility testing. MICs of the antibiotics tested were determined by broth microdilution in cation-adjusted Müller-Hinton broth (Trek Diagnostics Systems, East Grinstead, England) using Sensititre susceptibility EUST plates for *S. aureus,* EUVENC plates for enterococci, and EUVSEC plates for *E. coli* and *Pseudomonas* spp. (Trek). The list of antibiotics and the concentrations tested are presented in Table 1. *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, and *E. coli* ATCC 25922 were used as the control strains for MIC susceptibility testing. Results were always in the expected range. Testing for MICs was done for 164 isolates of interest. Twenty-two isolates of other enterococcal species and 10 isolates of *Pseudomonas* spp. were also tested but were not included in the result tables because epidemiological cutoff values (ECOFF) for interpretation were not available. Isolates were classified as susceptible or resistant according to ECOFF issued by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, version 3.0 (www.eucast.org). Because *E. faecalis* exhibited an intrinsic resistance to quinupristin-dalfopristin (Q-D), MIC data for this drug were not shown in Table 1 *(15, 23)*. Intrinsic resistances are adopted from EUCAST expert rules *(15)* and were excluded from the result tables except for gentamicin (GEN), to which only a low level of resistance is intrinsic in enterococci. For azithromycin (AZI), a value of >16 mg/liter was used for *E. coli* as a tentative ECOFF established from European Food Safety Authority (EFSA) data *(8)*.

RESULTS AND DISCUSSION

Bacteriological findings. Identification of 242 isolates by MALDI-TOF MS yielded 164 isolates of interest: *E. coli* ($n = 60$), *E. faecalis* ($n = 55$), *E. faecium* ($n = 6$),

TABLE 1. *MIC distribution for* Escherichia coli *(n = 60),* Enterococcus faecalis *(n = 55),* Pseudomonas aeruginosa *(n = 26), and* Staphylococcus aureus *(n = 9) isolates*

Antimicrobial agent	MIC (mg/liter)[a]																		Resistance
E. coli (n = 60)	0.016	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	(%)[b]
Ampicillin (AMP)							2	25	28					5					8
Azithromycin (AZI)*								1	34	25									ND
Cefotaxime (FOT)					60														0
Ceftazidime (TAZ)						60													0
Chloramphenicol (CHL)										57		1		2					5
Ciprofloxacin (CIP)	34	5	8	4	7		1				1								22
Colistin (COL)							60												0
Gentamicin (GEN)						33	26	1											0
Meropenem (MEM)		52			8														0
Nalidixic acid (NAL)									52	1			2	3	2				12
Sulfamethoxazole (SMX)										22	17	13						8	13
Tetracycline (TET)								49	1				5	5					17
Tigecycline (TGC)					60														0
Trimethoprim (TMP)					30	22	3						5						8
E. faecalis (n = 55)	0.016	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	
Ampicillin (AMP)						2	28	25											0
Chloramphenicol (CHL)										42	12		1						2
Ciprofloxacin (CIP)							11	41	3										0
Daptomycin (DAP)					1		8	31	15										0
Gentamicin (GEN)										17	34	2					1	1	4
Linezolid (LZD)						2	1	29	23										0
Teicoplanin (TEI)						54	1												0
Tetracycline (TET)							37	9					4	5					16
Tigecycline (TGC)			3	21	29	2													4
Vancomycin (VAN)							31	22	2										0
P. aeruginosa (n = 26)	0.016	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	
Azithromycin (AZI)												1	3	22					ND
Ceftazidime (TAZ)							12	14											0
Ciprofloxacin (CIP)	1		3	22															0
Colistin (COL)							4	22											0
Gentamicin (GEN)						2	20	4											0
Meropenem (MEM)			6	7	6		6		1										4
Nalidixic acid (NAL)												13	13						ND
Sulfamethoxazole (SMX)															11	9	6		ND
S. aureus (n = 9)	0.016	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	
Cefoxitin (FOX)									9										0
Chloramphenicol (CHL)										8				1					11
Ciprofloxacin (CIP)					7	2													0
Clindamycin (CLI)				8	1														0
Erythromycin (ERY)					1	8													0
Fusidate (FUS)						9													0
Gentamicin (GEN)							9												0
Kanamycin (KAN)									6				3						33
Linezolid (LZD)								9											0
Mupirocin (MUP)						9													0
Penicillin (PEN)				4	1				4										56
Quinupristin/Dalfopristin (Q-D)						9													0
Rifampin (RIF)	9																		0
Streptomycin (STR)										1	8								0
Sulfamethoxazole (SMX)													9						0
Tetracycline (TET)						6						3							33
Tiamulin (TIA)							9												0
Trimethoprim (TMP)								9											0
Vancomycin (VAN)							9												0

[a] Numbers are the number of isolates with the corresponding MIC. Open areas indicate the range of dilutions tested for each antimicrobial agent. Values above this range denote MICs greater than the highest concentration tested, and values at the lowest concentration tested denote MICs at or lower that lowest concentration tested. Vertical lines indicate epidemiological cut-offs (ECOFF) according to the European Committee on Antimicrobial Susceptibility Testing guidelines. Missing vertical lines indicate that no ECOFF was available. For azithromycin (*), a value of >16 mg/liter was used for *E. coli* as a tentative ECOFF (dotted line) established based on EFSA data *(8)*.

[b] ND, not determined.

TABLE 2. *Raw oyster, salmon, shrimp, and pangasius samples imported into Switzerland and positive for* Escherichia coli, Enterococcus faecalis, E. casseliflavus, Pseudomonas aeruginosa, *and* Staphylococcus aureus

Bacterial species	No. (%) of positive samples				
	Oyster ($n = 10$)	Salmon ($n = 11$)	Shrimp ($n = 11$)	Pangasius ($n = 12$)	Total ($n = 44$)
E. coli	4 (40)	3 (27)	7 (64)	10 (83)	24 (55)
E. faecalis	1 (10)	7 (64)	9 (82)	9 (75)	26 (59)
E. casseliflavus	1 (10)	0	4 (36)	0	5 (11)
P. aeruginosa	0	1 (9)	4 (36)	7 (58)	12 (27)
S. aureus	1 (10)	0	2 (18)	1 (8)	4 (9)

Enterococcus casseliflavus ($n = 8$), *P. aeruginosa* ($n = 26$), and *S. aureus* ($n = 9$). The other 78 isolates were identified as *Enterococcus gilvus* ($n = 6$), *Enterococcus thailandicus* ($n = 5$), *Enterococcus hirae* ($n = 4$), *Enterococcus mundtii* ($n = 3$), *Enterococcus phoenoculicola* ($n = 2$), *Enterococcus avium* ($n = 1$), *Enterococcus malodoratus* ($n = 1$), *Pseudomonas putida* ($n = 5$), *Pseudomonas monteilii* ($n = 1$), *Pseudomonas mosselii* ($n = 2$), *Pseudomonas oleovorans* ($n = 1$), *Pseudomonas otitidis* ($n = 1$), *Aeromonas veronii* ($n = 17$), *Aeromonas eucrenophila* ($n = 6$), *A. hydrophila* ($n = 2$), *Aeromonas bestiarium* ($n = 1$), *Aeromonas salmonicida* ($n = 1$), *Klebsiella pneumoniae* ($n = 1$), *Lactobacillus garvieae* ($n = 1$), *Lactobacillus lactis* ($n = 4$), *Providencia alcalifaciens* ($n = 1$), *Shewanella algae* ($n = 3$), *Shewanella putrefaciens* ($n = 4$), *Vagococcus fluvialis* ($n = 2$), *Vibrio cholerae* ($n = 1$), and unknown ($n = 2$).

The predominantly observed species isolated from all four samples types were *E. coli* and *E. faecalis,* found in 55 and 59% of the samples, respectively (Table 2). Less common was *P. aeruginosa,* found in 27% of the samples but not in oysters, followed by *E. casseliflavus* in 11% (in oysters and shrimps) and *S. aureus* in 9% of the samples but not in salmon (Table 2). *E. faecium* was rarely found, only twice in pangasius and once in oysters. In a testing program of ground waters in Switzerland, *E. faecalis* was more prevalent than *E. faecium,* leading to the hypothesis that in water *E. faecium* might not survive as well as *E. faecalis* (data not shown). Other enterococci were found in all sample types, such as *E. gilvus* in salmon and pangasius, *E. thailandicus* in oysters, shrimps, and pangasius, and *E. hirae* in oysters and shrimps.

Caution is advised concerning the origin of isolated bacteria. The tested samples were purchased at retail, and fecal indicators such as *E. coli* and enterococci might be a sign of animal and/or human fecal pollution of the aquaculture environment or could be acquired during processing because these bacteria are not part of the normal bacterial flora of fish and shellfish *(3, 16)*.

Antimicrobial resistance among bacterial isolates. The distribution of MICs and rate of resistance to antimicrobial agents are listed in Table 1 for 150 isolates. Because the small number of samples and bacterial isolates did not allow proper statistical comparison, we did not calculate confidence intervals for the proportion of resistant isolates. However, our data suggested several qualitative conclusions. In *E. coli,* several MDR isolates were found, including two strains resistant to seven antimicrobial agents (Table 3). Concerning quinolones, 22 and 12% of the isolates were resistant to ciprofloxacin (CIP) and nalidixic acid (NAL), respectively (Table 1). The rates of resistance were 17% to tetracycline (TET), 13% to sulfamethoxazole (SMX), 8% each to ampicillin (AMP) and trimethoprim (TMP), and 5% to chloramphenicol (CHL). None of the *E. coli* isolates were resistant to colistin (COL), cefotaxime, GEN, meropenem (MEM), ceftazidime (TAZ), and tigecycline (TGC). For all *E. coli* isolates, the MIC of AZI was ≤ 8 mg/liter, and theses isolates were considered susceptible. In *E. faecalis,* the highest resistance rate was against TET (16%) (Table 1). Lower resistance rates were found for CHL, GEN, and TGC. No resistance was found for AMP, CIP, daptomycin (DAP), linezolid (LZD), teicoplanin (TEI), and vancomycin (VAN). Isolates of *E. casseliflavus* were resistant only to TET. All six isolates of *E. faecium* were resistant to Q-D, and some were also resistant to DAP and TET. None of the *E. faecium* isolates were resistant to AMP, CHL, CIP, GEN, LZD, TEI, TGC, and VAN. Of the other enterococcal species, MICs of 4 and 2 mg/liter for Q-D were found for 64 and 9% of the isolates, respectively, probably indicating resistance because intrinsic resistance to Q-D for these enterococci has not been described *(15, 23)*. For all *P. aeruginosa* isolates, MICs of AZI were ≥ 32 mg/liter. MICs of NAL and SMX (no ECOFF available) are listed in Table 1. None of the *P. aeruginosa* isolates were resistant to TAZ, CIP, COL, and GEN (Table 1). For one *P. aeruginosa* isolate (from salmon, Table 1) and one *P. monteilii* isolate (from oyster), the MICs of MEM were 4 mg/liter and those for imipenem were 8 and 2 mg/liter, respectively. The *P. aeruginosa* isolate was sensitive to TAZ and cefepime, and for the *P. monteilii* isolate, the MICs of TAZ and cefepime were 4 and 2 mg/liter, respectively. Screening tests for detection of carbapenemases were negative for both isolates (Rapidec Carba NP, bioMérieux). In *S. aureus*, the highest resistance rates were to penicillin (56%), TET (33%), and kanamycin (33%) (Table 1). One isolate was resistant to CHL. This finding is in contrast to the results of a Swiss monitoring program *(10),* in which CHL resistance was never found among the methicillin-resistant *S. aureus* (MRSA) between 2010 and 2014. None of the *S. aureus* isolates were resistant to cefoxitin, CIP, clindamycin, erythromycin, fusidate, GEN, LZD, mupirocin, Q-D,

TABLE 3. *Multidrug resistance of* Escherichia coli, Enterococcus faecalis, *and* Staphylococcus aureus *found in pangasius (P) and shrimps (S) imported into Switzerland*

Resistance (fold)	Sample *n*	Type	Antimicrobial agent and susceptibility results[a]
E. coli			AMP AZI* CHL CIP COL FOT GEN MEM NAL SMX TAZ TET TGC TMP
7	2	S	■ ■ ■ ■ ■ ■
5	1	S	■ ■ ■ ■ ■
5	1	S	■ ■ ■
4	1	S	■ ■ ■ ■
3	1	P	■ ■ ■
E. faecalis	n	type	AMP CHL CIP DAP GEN LZD TEI TET TGC VAN
3	1	S	■ ■ ■
S. aureus			CHL CIP CLI ERY FOX FUS GEN KAN LZD MUP PEN Q-D RIF SMX STR TET TIA TMP VAN
4	1	P	■ ■ ■ ■
3	1	S	■ ■ ■

[a] Shaded areas indicate resistance. Susceptibility results are based on epidemiological cut-offs (ECOFF) according to the European Committee on Antimicrobial Susceptibility Testing guidelines. For AZI(*), a value of >16 mg/liter was used as a tentative ECOFF established based on EFSA data by the European Reference Laboratory for Antimicrobial Resistance (Lyngby, Denmark). AMP, ampicillin; AZI, azithromycin; CHL, chloramphenicol; CIP, ciprofloxacin; COL, colistin; FOT, cefotaxime; GEN, gentamicin; MEM, meropenem; NAL, nalidixic acid; SMX, sulfamethoxazole; TAZ, ceftazidime; TET, tetracycline; TGC, tigecycline; TMP, trimethoprim; DAP, daptomycin; LZD, linezolid; TEI, teicoplanin; VAN, vancomycin; CLI, clindamycin; ERY, erythromycin; FOX, cefoxitin; FUS, fusidate; KAN, kanamycin; MUP, mupirocin; PEN, penicillin; Q-D, quinupristin-dalfopristin; RIF, rifampin; STR, streptomycin; TIA, tiamulin.

rifampin, streptomycin, SMX, tiamulin, TMP, and VAN (Table 1).

AZI may be used to treat human infections with MDR gram-negative bacteria, in particular invasive *Salmonella* strains. It is not known to what extent such newer, long-acting modern macrolides authorized in veterinary medicine for food-producing animals select for resistance to macrolides in *E. coli* and *Salmonella*. Therefore, monitoring of resistance to AZI recently became mandatory for states of the European Union *(7)*. It will now be possible to gather data to establish MIC distributions and to determine the ECOFF. In the meantime, a value of >16 mg/liter was used as a tentative ECOFF established from EFSA data *(8)*.

MDR among samples. Patterns of MDR are shown in Table 3. No MDR isolates from oysters and salmon were found. Of the 11 shrimp samples, 5 (45%) harbored MDR *E. coli,* 1 (9%) had MDR *E. faecalis,* and 1 (9%) had MDR *S. aureus*. None of the *P. aeruginosa* and *E. casseliflavus* isolates were MDR. One sample each (8%) of the 12 pangasius samples harbored an MDR *E. coli* and an MDR *S. aureus,* respectively. None of the *P. aeruginosa* and *E. faecalis* isolates were MDR.

To summarize, all sample types harbored antimicrobial resistant bacteria. MDR (mainly among *E. coli* isolates) was found in 64% of shrimp samples and 17% of pangasius samples. The MDR *S. aureus* strain from a shrimp sample, which was resistant to aminoglycosides, β-lactams, and tetracyclines, originated from an organic production system (in Indonesia), though human contamination cannot be excluded. To detect MRSA, selective enrichment would be a more sensitive method because the prevalence of these strains seemed to be low. In another study, 2 (20%) of 10 pangasius samples tested positive for ESBL-producing *E. coli,* which were isolated after selective enrichment (data not shown). This finding highlights the importance of using more sensitive selective enrichment methods for the reliable detection of bacteria with special resistance characteristics, such as MRSA or bacteria producing ESBL or carbapenemases. Attention also should be paid to the number of presumptive colonies isolated per target organism and sample. In this study, up to three presumptive isolates were selected. If only one isolate had been evaluated, several single-resistance and MDR isolates would have been missed, as indicated by the fact that the three isolates evaluated often had different antimicrobial resistance patterns.

The need for surveillance and monitoring of carbapenem resistance in food and other nonhuman sources was also postulated by Woodford et al. *(26)*. Monitoring of this type of resistance is particularly important in foods such as oysters, which are eaten raw and do not undergo processing steps to eliminate resistant bacteria before consumption. Resistance to carbapenems needs particular attention because this antimicrobial agent is a drug of last resort, listed as a critically important antimicrobial agent by the World Health Organization *(27)*.

The reasons for the generally lower burden of resistant bacteria in oysters and salmon, compared with shrimps and pangasius, are unknown. Differences in water temperature between the northern Atlantic and the warmer waters where shrimps and pangasius are produced might play a role. Fecal contamination, known to be present in higher concentrations in Asian coastal waters *(3)* than in the northern Atlantic, also could have an influence.

Conclusions with respect to future monitoring programs. Because of its high prevalence, *E. coli* was

considered a possible target for monitoring of pangasius, shrimps, and oysters (Table 2). This pathogen is less suitable for salmon, in which the *E. coli* prevalence was only moderate (27%). Concerning enterococci, high *E. faecalis* prevalences of 64 to 82% in salmon, shrimps, and pangasius suggest that these products should be monitored, but oysters are not a problem. *P. aeruginosa* could be taken into consideration for monitoring of shrimps and pangasius, in which prevalences of 36 and 58% were recorded. Because of the observed low prevalence of *S. aureus, E. casseliflavus,* and *E. faecium,* these bacterial species were considered unsuitable targets for monitoring purposes.

E. coli, E. faecalis, and MRSA are already part of programs used to monitor foods such as meat *(10)*. Our results indicate that these three bacterial species might also be useful for monitoring the microbial status of certain fish and seafood, particularly products imported from areas suspected to be contaminated. Antimicrobial resistance data and trends for these products would be useful because comparisons with available data for meat would be possible. Monitoring for bacteria in fish and seafood might provide information for risk management measures and uncover gaps in knowledge that warrant further investigation.

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