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Comparison of antimicrobial resistance phenotypes and genotypes in enterotoxigenic *Escherichia coli* isolated from Australian and Vietnamese pigs

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ABSTRACT

This study aimed to compare the antibiogram phenotype and carriage of antimicrobial resistance genes (ARGs) of 97 porcine multidrug-resistant (MDR) enterotoxigenic *Escherichia coli* (ETEC) isolates obtained from Vietnam and 117 porcine MDR-ETEC obtained from Australia, two countries with different antimicrobial regulation systems. An antimicrobial resistance index (ARI) was calculated to quantify their potential significance to public health. Both Vietnamese and Australian isolates had moderate to high levels of resistance to commonly used antibiotics (ampicillin, tetracycline and sulphonamides). None of the Australian isolates were resistant to fluoroquinolones or third-generation cephalosporins and none possessed associated plasmid-mediated ARGs. However, 23.1% of Australian isolates were resistant to gentamicin owing to ARGs associated with apramycin or neomycin resistance [e.g. *aac(3)-IV*] that impart cross-resistance to gentamicin. Whilst Vietnamese isolates carried aminoglycoside ARGs, 44.4% of commercial pig isolates were resistant to gentamicin in comparison with 0% of village pig isolates. The plasmid-mediated fluoroquinolone ARG *qnrB* was commonly detected in Vietnamese isolates (52.3% commercial, 44.1% village), but phenotypic resistance was low (3.2% and 11.8%, respectively). The mean ARI for Vietnamese isolates (26.0) was significantly different ($P < 0.001$) from the mean ARI for Australian isolates (19.8), primarily reflecting fluoroquinolone resistance in the former collection. This comparison suggests the effectiveness of regulations that slow the dissemination of ‘critical’ resistance by restricting the availability of important classes of antimicrobials.

1. Introduction

Enterotoxigenic *Escherichia coli* (ETEC) is an important pathogen in swine production, causing neonatal diarrhoea and post-weaning diarrhoea in young piglets. Although a wide range of therapeutic products are effective, the availability of specific drugs varies between countries [1,2] owing to regulatory differences for use in animals. The most commonly used antimicrobials include ampicillin, neomycin, apramycin, spectinomycin, colistin, zinc oxide and potentiated sulphonamides [3]. Long-standing reliance on these drugs has resulted in selection for antimicrobial resistance, and multidrug resistance has become common in porcine ETEC [4–7].

Australian regulations governing antimicrobial use in livestock are conservative [8], with a restricted range of antimicrobials registered [9]. Neither fluoroquinolones nor gentamicin can be administered [10], and the third-generation cephalosporin ceftiofur has strict label constraints. However, it can be used ‘off-label’ to treat colibacillosis caused by multidrug-resistant (MDR) strains of *E. coli* [5]. Fluoroquinolone drugs and the third-generation cephalosporins are regarded as ‘critically important’ in human medicine and should be considered as drugs of last resort in food animals [11]. The most recent survey into Australian swine herds reported strong reliance on antimicrobials not considered to be as critically important to human health (such as penicillins, tetracyclines and sulphonamides) [12]. Nevertheless, the same study revealed that 25% of herds had used ceftiofur in the previous year, suggesting difficulties in managing infections such as MDR-ETEC.

In a recent study of Australian porcine ETEC [4], no resistance to ceftiofur or fluoroquinolones was found. However, a large number of isolates were MDR to 'lower importance' antimicrobials. Some isolates were resistant to the aminoglycosides, apramycin and neomycin (ca. 30% for each), presumably as a consequence of their recent use in ETEC management.

Aminoglycoside resistance may pose a potential risk as some resistance genes can impart cross-resistance to gentamicin, which is still highly valued in human medicine [13]. Whilst controlled use of antimicrobials in Australian pigs has probably slowed the onset of resistance to some 'critical' drugs [4], it may come at the cost of more rapid selection of resistance to other groups such as the aminoglycosides. One approach for studying this is to compare the resistance attributes of ETEC isolates originating from different regulatory environments.

Vietnam is representative of many developing countries. It has high levels of antimicrobial use both in humans and animals and resistance among bacterial pathogens is widespread [1]. Use of antimicrobials in commercial pig production is largely unregulated and access to veterinary advice is limited. In village-based enterprises, pigs may be treated with any available antibiotics [14,15]. Consequently, in Vietnam and countries with similar regulation systems, there is potential for rapid emergence of antimicrobial resistance to a wide variety of drugs. However, whilst one study has focused on resistant phenotypes in pathogenic *E*. *coli* isolates from swine in

Vietnam [1], no studies have been conducted to correlate the drug resistance phenotype with genotypes.

Pigs in Australia are virtually all raised under the same system of intensive production common to the developed world, albeit with a conservative approach to antimicrobial registration. Pigs in Vietnam are managed in two distinctly different production systems, which are both relatively unregulated with regard to antimicrobial use: village pigs raised by smallholder farmers; and commercial pigs raised using an intensive approach similar to Australia.

In a previous study, Smith et al. characterised a collection of Australian porcine ETEC isolates to determine the frequency of occurrence of single resistance attributes and associated antimicrobial resistance genes (ARGs), allowing an antimicrobial resistance index (ARI) to be attributed to each isolate [4]. The current study applied the same methodology to a collection of ETEC isolates obtained from village and commercial pigs from Vietnam and then compared these results with the data previously obtained from the Australian ETEC porcine collection, with the aim of investigating relationships between levels of regulatory control of antimicrobial use with phenotypic and genotypic resistance levels amongst production porcine isolates.

2. Materials and methods

2.1. Bacterial isolates

A total of 97 MDR-ETEC isolates (2001) were obtained from Vietnamese pig faecal samples as described previously [1]. Animals' symptoms were consistent with porcine colibacillosis. In total, 63 and 34 isolates were collected from commercial piggeries and village piggeries, respectively, in northern Vietnam.

Veterinary diagnostic laboratories throughout Australia provided 117 MDR-ETEC isolates (1999–2005), as described in a previous study [4]. The isolates originated from Queensland (*n* = 52), Victoria (*n* = 28), South Australia (*n* = 27), Western Australia (*n* = 8) and New South Wales (*n* = 2). All isolates were obtained from the faeces or intestine of pigs with symptoms of post-weaning diarrhoea and were stored in brain–heart infusion broth (Oxoid Australia Pty Ltd., Thebarton, SA, Australia) plus 20% glycerol (ChemSupply Pty Ltd., Gillman, SA, Australia) at –80 °C and as freeze-dried specimens.

2.2. Antimicrobial susceptibility testing

The ETEC collection was screened for resistance to 12 antimicrobial agents from seven different chemical classes (ampicillin, ceftiofur, gentamicin, apramycin, neomycin, spectinomycin, streptomycin, florfenicol, chloramphenicol, enrofloxacin, tetracycline and trimethoprim/sulfamethoxazole) using the disk diffusion susceptibility testing method performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines

[16]. Isolates with resistance to three or more antimicrobials by disk diffusion were then subjected to minimum inhibitory concentration (MIC) susceptibility testing (using the same antimicrobial agents listed above) performed by the broth microdilution method in 96-well microtitre plates (Sarstedt Australia Pty Ltd., Technology Park, SA, Australia) as described in the CLSI standards manual [16]. Inhibition zones and MICs were interpreted using CLSI recommended inhibition breakpoints for enteric pathogens where available. As there are no CLSI breakpoints for florfenicol and ceftiofur applicable to *E. coli* of animal origin, breakpoints for florfenicol (≥16 μg/mL) and ceftiofur (≥8 μg/mL) were sourced from the Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP) [17,18]. Inhibition zone sizes used for florfenicol [resistant (R) ≤14 mm, susceptible (S) ≥19 mm] and ceftiofur (R ≤17 mm, S ≥21 mm) were CLSI breakpoints established for bovine respiratory disease isolates [16]. Neomycin (≤12 mm and ≥16 μg/mL) (Zoetis, West Ryde, NSW, Australia) and apramycin (≤10 mm and ≥32 μg/mL) (Bayer Australia Ltd., Pymble, NSW, Australia) breakpoint information was obtained directly from the respective manufacturers. *Escherichia coli* reference strain ATCC 25922 was used as a control.

2.3. Identification of antimicrobial resistance genes by PCR

Single PCR was used to detect the presence of 32 ARGs in the Vietnamese isolate collection as described previously [4]. Single PCR was also use to detect the presence of the plasmid-mediated quinolone resistance genes *qnrA*, *qnrB* and *qnrS* in all isolates [19]. All targeted ARGs have previously been identified in enteric Gram-negative organisms and encode resistance or reduced susceptibility to one or more of the 12

antimicrobial agents tested. The *int* primers were used to detect the presence of class 1 integrons, with positive amplicons subjected to a second PCR using the *intVR* primers [20].

2.4. Antimicrobial resistance index calculation

An ARI was calculated for each Vietnamese isolate using a previously described algorithm developed to calculate the ARI of the Australian isolates [4]. Briefly, the ARI accounts for the measured extent of phenotypic resistance to each of 12 drugs used to assess phenotypic resistance, the antimicrobial resistance genes present, and the number and type of integrons present. Within the index, phenotypic or genetic resistance to drugs of public health significance is given extra weighting. Importance weightings were based on the Expert Advisory Group on Antimicrobial Resistance (EAGAR) recommendations [21]. Comparisons between populations (Australian and Vietnamese commercial and village piggeries) in the proportion of isolates testing positive to various traits were assessed for significance using Fisher's exact test. The ARI for each collection of isolates were graphically summarised and *t*-tests were used to assess the equality of means between source populations. All analysis was performed in Stata SE v.13.0 (Stata Corp., College Station, TX).

3. Results

The occurrence of phenotypic resistance by MIC and associated ARGs for Australian and Vietnamese ETEC isolates are shown in Table 1. No isolates were resistant to ceftiofur and no associated ARGs were detected.

3.1. Vietnamese isolates

A small proportion of Vietnamese ETEC isolates were resistant to enrofloxacin (6.2%, *n* = 6; MIC range 32–64 μg/mL). The plasmid-mediated fluoroquinolone ARG *qnrB* was identified in 49.5% of isolates (*n* = 48), including all fluoroquinolone-resistant isolates. The remaining *qnrB*-positive isolates all had fluoroquinolone MICs below the susceptible breakpoint of ≤1 μg/mL (MIC range <0.25–0.5 μg/mL). Gentamicin resistance was observed in 28.9% of isolates (*n* = 28), and aminoglycoside ARGs capable of causing gentamicin resistance were identified in 85.7% (*n* = 24) of the resistant and 34.8% (n = 24) of the susceptible isolates. Over one-half of the isolates (54.6%; *n* = 53) exhibited resistance to florfenicol; however the *floR* gene was not detected. The prevalence of integrons in the Vietnamese isolate collection was 97.9% (*n* = 95). All integrase-positive isolates had at least one integron-associated gene cassette and four isolates possessed two gene cassettes.

3.2. Comparison of Vietnamese commercial and village piggeries

The prevalence of enrofloxacin resistance differed between commercial and village piggeries, but not significantly (3.2% vs. 11.8%; *P* = 0.18). The *qnrB* gene was more

frequent in Vietnamese commercial piggery isolates than village isolates (Table 1). Ampicillin resistance and associated ARGs were prevalent. Approximately one-half (49.2%) of the commercial piggery isolates carried $bla_{TEM.}$ Village piggery isolates had a higher diversity and pairing of β-lactamase genes; bla_{TEM} was often paired with bla_{OXA} and once with bla_{SHV}.

All Vietnamese isolates displaying phenotypic resistance to gentamicin and/or neomycin originated from commercial piggeries. However, several isolates from village pigs carried gentamicin ARGs even though all of these yielded gentamicin MICs below the resistant breakpoint, with the highest value being 2 μg/mL. All aminoglycoside ARGs associated with gentamicin resistance were carried separately in the village piggery isolate collection, whereas the commercial piggery isolates often carried them in pairs, the most common pairing being *aac(3)-II* and *aac(3)-IV*, which may explain the difference in aminoglycoside resistance between the two production systems. Resistance to chloramphenicol was more common in commercial pig isolates compared with those from village pigs (69.8% vs. 47.1%; $P = 0.03$), however the difference for florfenicol was not significant (60.3% vs. 44.1%; $P = 0.14$). The ARGs *cmlA* and *catI* were detected in both groups of Vietnamese isolates. Three isolates from commercial pigs carried both *cmlA* and *catI*.

Integrons were prolific in Vietnamese isolates with 93% ($n = 59$) of commercial pig isolates and 97.1% ($n = 33$) of village pig isolates positive for integrase. Every isolate

that possessed two gene cassettes within the variable region originated from the village pig collection.

The mean ARI score for the Vietnamese commercial pig isolates was 28.7 (range 12–46). By contrast, Vietnamese village pig isolates had a significantly ($P < 0.001$) lower mean ARI score of 21.2 (range 10–29.5), even though they had a higher prevalence of fluoroquinolone resistance. The most notable commercial pig isolates from a public health perspective (the 15% of isolates that had the highest ARI scores and that were all positive for *qnrB*) had ARI scores ranging from 41.5 to 46.0, in comparison with village pig isolates for which they ranged from 27.5 to 29.5 (Fig. 1). The difference in ARI scores between the commercial pig and village pig isolates can be primarily attributed to differences in gentamicin resistance. Here the commercial pig isolates often had MICs >16 μg/mL, with some having MICs >128 μg/mL. These gentamicin-resistant isolates had a strong association with apramycin resistance and always possessed *aac(3)-IV*.

3.3. Vietnamese and Australian enterotoxigenic Escherichia coli collection comparison

Vietnamese isolates demonstrated higher MICs and associated ARG possession for enrofloxacin and florfenicol. Enrofloxacin resistance and *qnr* ARGs were totally absent in the Australian collection. The ARG *floR* was absent in both collections, but florfenicol resistance was also very low in the Australian collection in comparison with the Vietnamese collection (Table 1). The differences in gentamicin and apramycin resistances were marginal. However, neomycin resistance was much higher in the

Australian collection. The Australian collection had a higher frequency of the aminoglycoside ARGs *aac(3)-IV* and *aph(3')-I*, but lacked *aac(3)-II* and *aac(3)-III*.

Ampicillin resistance was less prevalent in the Australian collection. The only β-lactamase gene detected was bla_{TEM}, which was found at a comparable frequency to the Vietnamese collection. Approximately one-half of the Australian collection carried an integron, but these isolates were more likely to carry two gene cassettes.

With regard to ARI scores, the Vietnamese collection (as a whole) had a significantly higher mean score of 26.0 (isolates ranged from 10 to 46) compared with the Australian collection mean score of 19.8 (isolates ranged from 6 to 36) (Fig. 1). The difference in ARI scores can be directly attributed to the presence of enrofloxacin resistance and the high frequency of *qnrB* in the Vietnamese isolates as well as the much higher prevalence of florfenicol resistance.

3.4. Prevalence of multiple resistance phenotypes

Vietnamese commercial piggery isolates demonstrated the highest incidence of more extended MDR phenotypes, with distribution skewed towards a higher proportion of isolates resistant to seven or more antimicrobials. Less than 10% of these isolates were resistant to four antimicrobials, with 15% resistant to ten antimicrobials (Fig. 2). The largest proportion of Vietnamese village isolates (>50%) was resistant to six or seven different antimicrobials. The occurrence of multiple antimicrobial resistances for the

Australian collection was more normally distributed, with the bulk of isolates displaying between three and seven extended MDR phenotypes.

4. Discussion

The ARI score was created and used here to simplify evaluation of resistance through use of a single measure to distinguish between collections of porcine ETEC isolates with different antimicrobial selection pressures. This investigation found that the prevalence of multiple resistances, the degree of resistance and the frequency of ARGs were more common in Vietnamese compared with Australian ETEC isolate collections. This was also true for the isolates from Vietnamese commercial piggeries compared with village piggeries.

In general, compared with Australian isolates, the Vietnamese isolates carried more ARGs, were more frequently resistant to critically important drugs and were more likely to have MICs one or two times greater than the resistance breakpoints. Specifically, the presence of fluoroquinolone resistance and *qnrB*, in addition to a higher prevalence of florfenicol resistance, elevated the Vietnamese isolates' ARI. Vietnamese isolates also had a much higher proportion of integron-associated ARGs and a greater array of amoxicillin-associated ARGs.

The commercial Vietnamese piggery collection had a higher level of resistance and a higher frequency of ARGs for almost all drug classes compared with the village piggeries, suggesting that commercial animals had more exposure to a greater array of

antimicrobials. This could be due to a range of factors/practices in commercial piggeries, e.g. more regular prophylactic use rather than therapeutic treatment of individual animals, greater antimicrobial choice in an environment predisposing to ETEC infection, and greater capacity for resistance emergence and dissemination.

The higher frequency of enrofloxacin resistance and the presence of *qnrB* in village piggeries indicates that smallholder farmers in rural areas are probably using fluoroquinolones more frequently as therapeutics. Unfortunately, no official antimicrobial usage data are available in Vietnam; however, livestock antimicrobial usage is typically at the smallholder farmer's discretion [14] and reports state that fluoroquinolones may be overprescribed or misused both in human and veterinary medicine [22,23]. The high frequency of *qnrB* in susceptible isolates may indicate that antimicrobial selective pressure is promoting dissemination of transferable plasmid-mediated resistance determinants, which may reduce susceptibility to fluoroquinolones and facilitate the development of stepwise point mutations in chromosomal target genes [24].

Vietnamese isolates showed a high prevalence of florfenicol resistance compared with Australian isolates. Florfenicol was released in 2003 for use in pigs in Australia [25] and, to the best of our knowledge, was not available in Vietnam at the time of sampling. Although *floR* was not detected in either collection, the high prevalence of florfenicol resistance in Vietnamese ETEC isolates may be associated with the *cmlA* gene, which may be related to historic use of chloramphenicol in livestock and/or co-selection [26].

Within the Vietnamese ETEC collection, resistance to gentamicin and neomycin was only present in the commercial piggery isolates. This may be evidence that commercial piggeries take a more regimented approach by using gentamicin, neomycin and/or apramycin to control outbreaks of colibacillosis during crucial growth periods. Gentamicin cannot be used in Australian livestock; however, it appears that the use of apramycin and neomycin is increasing resistance to gentamicin via ARGs capable of imparting cross-resistance [e.g. apramycin and the ARG *aac(3)-IV*] [27]. The Australian ETEC collection lacked the same aminoglycoside ARG diversity identified in the Vietnamese ETEC collection; however, the prevalence of resistance to gentamicin in Australian ETEC was only 6% below that determined for Vietnamese ETEC, which were isolated from animal production systems with little or no restriction on gentamicin use.

The absence of ceftiofur resistance and its associated ARGs in isolates from Australia may indicate that at the time of sampling, selection pressure from third-generation cephalosporin use was not sufficient to promote selection and dissemination of CTX-M- or AmpC-containing plasmids. To the best of our knowledge, third-generation cephalosporins were not commercially available in Vietnam at the time of sampling.

The difference between the phenotypic resistances displayed and the associated ARGs suggests that possession of a gene does not always accurately reflect the organism's resistance phenotype. For example, chloramphenicol, tetracycline and sulphonamide/trimethoprim ARGs were more prevalent compared with the correlated phenotypic resistances within the Australian collection, whereas actual phenotypic

resistance was more commonly observed in the Vietnamese collection. Whilst it is possible that the ARGs detected may be mutated and/or non-functional, inducible or not expressed, the observed phenotypic resistance could be a product of additional resistance mechanisms such as multidrug efflux pumps, specific mutations in outer membrane porins, or other unidentified ARGs.

The described isolate collections and associated analyses represent a valuable baseline for future benchmarking to confirm whether ARI scores rise or decline in response to changing antimicrobial usage patterns and production systems. There are several antimicrobial resistance surveillance groups around the world, such as the Australian Group on Antimicrobial Resistance (AGAR) [28], the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) [29] and the Global Antibiotic Resistance Partnership (GARP), which recently included Vietnam [14]. Integration of these types of programmes to monitor human and veterinary antimicrobial use and resistance is vital to effective resistance management. The main limitation to such global monitoring approaches is standardising sampling and testing approaches. For instance, in the current study the Vietnamese isolates were collected over a period of 6 months, whereas the Australian collection was obtained over a 6-year period. Other limitations are the range of ARGs targeted for each antimicrobial chosen. It is impossible to screen for all known ARGs and, like other transmissible genes, ARG frequencies may change in response to different selective pressures. The current study chose the most frequently identified ARGs, linked to transferable plasmids, in

pathogenic *E. coli*. Vigilant longitudinal monitoring programmes could be effective in following these changes.

This work suggests a clear example of how the regulation of antimicrobials in animal production can limit the emergence and/or dissemination of corresponding resistance mechanisms. This is particularly relevant to highly integrated or intensive production systems, where antimicrobial use is high and management/biosecurity practices allow for dissemination of resistant bacteria. Lack of regulation in such production systems is also a particular issue for the generation of MDR phenotypes or resistance to drugs of importance to public health. Continued reliance on antimicrobial use for colibacillosis in pigs potentially reduces the effectiveness of registered drugs as treatment options. In a poorly regulated environment, this forces the use of later generation or more critically important drugs. 'Extra-label' use of ceftiofur in Australian MDR-ETEC infections is evidence of this. Antimicrobial resistance is a worldwide problem and a globally consistent approach to regulation of antimicrobial use is needed to slow the inevitable rise of multidrug resistance. Regulation alone is unlikely to be effective and probably needs to be accompanied by improvement in animal management systems, antimicrobial stewardship, statutory enforcement, and continued application and development of antimicrobial alternatives. Meanwhile, surveillance of antimicrobial use and resistance in high-use environments such as intensive piggeries is crucial.

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Fig. 1. Distribution of the antimicrobial resistance (AMR) index for enterotoxigenic *Escherichia coli* isolates obtained from Australian pigs and from commercial and village pigs in Vietnam. Isolates represented by a cross (X) carry *qnrB*, whereas those represented by an open circle (O) do not. Horizontal lines are the mean index ± 1.96 standard deviations for each population grouping.

Fig. 2. Distribution of multiple resistance in enterotoxigenic *Escherichia coli* isolates from pigs in (a) Australian herds, (b) Vietnamese commercial herds and (c) Vietnamese village herds. Phenotypic resistance count indicates the number of drugs for which isolates expressed resistance out of 12 that were evaluated.

Table 1

Occurrence of phenotypic resistance by minimum inhibitory concentration (MIC) and associated antimicrobial resistance genes (ARGs) for Australian and Vietnamese enterotoxigenic *Escherichia coli* isolates

Antimicrobial agent	Resistance trait [a]	Percent of isolates with each resistance trait			
		Australian (*n* = 117)	Vietnamese commercial (*n* = 63)	Vietnamese villages (*n* = 34)	Total Vietnamese isolates (*n* = 97)
Ceftiofur	MIC	0	0	0	0
	AmpC	0	0	0	0
	bla$_{CTX-M}$	0	0	0	0
Ampicillin	MIC	56.4	100.0	85.3	94.8
	bla$_{TEM}$	44.4	49.2	41.2	46.4
	bla$_{SHV}$	0	7.9	23.5	13.4
	bla$_{OXA}$	0	4.8	23.5	11.3
Enrofloxacin	MIC	0	3.2	11.8	6.2
	qnrB	0	52.3	44.1	49.5
Florfenicol	MIC	13.7	60.3	44.1	54.6
	floR	0	0	0	0
Chloramphenicol	MIC	33.3	69.8	44.1	60.8
	catI	25.6	9.5	14.7	11.3
	catII	0	0	0	0
	cmlA	42.7	50.8	8.8	36.1
Gentamicin	MIC	23.1	44.4	0	28.9
	aac(3)-II	0	20.6	5.9	15.5
	aac(3)-III	0	7.9	14.7	10.3
Apramycin	MIC	27.4	34.9	26.5	32.0
	aac(3)-IV	45.3	36.5	11.8	27.8

Neomycin	MIC	45.3	19.0	0	12.4
	aph(3')-I	31.6	15.9	5.9	12.4
Streptomycin	MIC	87.9	96.8	85.3	92.8
Spectinomycin	MIC	77.8	73.0	29.4	57.7
	ant(3')-I	93.2	88.9	79.4	85.6
Tetracycline	MIC	81.2	100.0	100.0	100.0
	tetA	41.9	49.2	64.7	54.6
	tetB	29.9	0	0	0
	tetC	16.2	3.2	5.9	4.1
SXT	MIC	52.1	98.4	85.3	93.8
	sulI	65.8	55.6	55.9	55.7
	sulII	21.4	22.2	32.4	25.8
	dhfrI	2.6	6.3	26.5	13.4
	dhfrV	31.6	57.1	94.1	70.1
	dhfrXII	30.8	23.8	5.9	17.5

[a] MIC refers to phenotypic resistance by MIC; AmpC refers to five primer sets targeting different *ampC* genes; $bla_{\text{CTX-M}}$ refers to four primer sets targeting CTX-M variants. The ARGs *aac(3)-I*, *aph(3')-II*, *qnrA*, *qnrS* and *catIII* were not detected.