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Review

Beta-lactam resistance among *Enterobacteriaceae* in Cambodia: The four-year itch

Yannick Caron[a,1], Rattanak Chheang[a,1], Nop Puthea[a], Meng Soda[a], Sébastien Boyer[b], Arnaud Tarantola[c], Alexandra Kerléguer[a,*]

[a] *Medical Biology Unit, Institut Pasteur du Cambodge, Phnom Penh, Cambodia*
[b] *Medical Entomology Platform, Institut Pasteur du Cambodge, Phnom Penh, Cambodia*
[c] *Epidemiology and Public Health Unit, Institut Pasteur du Cambodge, Phnom Penh, Cambodia*

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ABSTRACT

Although antibiotics are too often used inappropriately in Cambodia, published data on antimicrobial resistance in this country are scarce. Epidemic dissemination and the transfer of resistance genes to other bacterial species put the population at risk. The aim of this study was to evaluate the frequency and characteristics of extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* (ESBL-E) isolated in consecutive samples tested at Institut Pasteur du Cambodge over a 4-year period (2012–2015). Antimicrobial susceptibility testing was performed by disk diffusion on agar technique and the results were read automatically using an OSIRIS system. The Etest was used to determine minimum inhibitory concentrations (MIC) for some resistance phenotypes. The strain most commonly identified was *Escherichia coli* (63.9%). The proportion of ESBL-E increased gradually over the study period, from 23.8% to 38.4%. ESBL was detected in 42.7% of the *E. coli* strains and 33.7% of all *Klebsiella pneumoniae* isolated. The proportion of ESBL-producing *E. coli* increased significantly from 28.9% in 2012 to 48.2% in 2015, while the increase for *K. pneumoniae* remained non-significant. Multidrug resistance was high in this Cambodian series, with some strains displaying resistance to all antibiotics available in the country. There is currently no established system for the surveillance of antimicrobial resistance in Cambodia. Collecting samples from clinical settings throughout the country is critical to assess the impact of antimicrobial drug use in patients in Cambodia and in the Mekong Region.

Contents

* Corresponding author at: Medical Biology Unit, Institut Pasteur du Cambodge, BP 583, Phnom Penh, Cambodia.
E-mail address: akerleguer@pasteur-kh.org (A. Kerléguer).
[1] These authors contributed equally to the study.

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Introduction

Antibiotic resistance is on the rise worldwide and is associated with severe morbidity, mortality, increased healthcare-related costs, and a reduction in gross domestic product (GDP) globally (O'Neill, 2015). The frequency of extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* (ESBL-E) has been increasing steadily worldwide since the early1980s (Knothe et al., 1983; Pitout et al., 2008). Enzymes inactivating carbapenems have subsequently appeared and have disseminated rapidly across the globe, leading to increased therapeutic challenges (Osano et al., 1994).

ESBL are beta-lactamases capable of conferring bacterial resistance to penicillins, first-, second-, and third-generation cephalosporins (3GC), and aztreonam (except cephamycins and carbapenems) by hydrolysis of these antibiotics. They are inhibited by beta-lactamase inhibitors such as clavulanic acid (Philippon, 2013).

The spread of antibiotic resistance is attributed principally to the overuse and/or inappropriate use of antibiotics for prophylaxis and the treatment of infections in humans and animals, or as agricultural growth-promotants (Haenni et al., 2014). The horizontal transmission of antibiotic resistance through the diffusion of clonal strains or antibiotic resistance genes is the most significant means of emergence and spread, especially due to selective pressure (Ito et al., 1995).

In Cambodia, antibiotic use is often inappropriate, widespread, and unregulated, but few data are available on ESBL-E and other bacterial antimicrobial resistance (Ruppe et al., 2009). The aim of this study was to contribute to the estimation of the burden of ESBL-E in Cambodia.

Materials and methods

Location and type of study

The study objectives were (1) to evaluate the frequency of ESBL-E; (2) to estimate the frequency of fecal carriage; and (3) to estimate the proportions of ESBL-E isolated in samples originating from hospitals and/or the community (non-hospitalized patients). A retrospective study was performed using data from all consecutive blood, stool, pulmonary, pus, genito-urinary, and other body fluid samples tested between January 1, 2012 and December 31, 2015 in the microbiology unit of the clinical laboratory at the Institut Pasteur du Cambodge, a reference microbiology laboratory in Cambodia. These samples all originated from Phnom Penh and were collected from Hospital Calmette (a national reference hospital for adults), Choy Ray Hospital (a community clinic), and walk-in patients who self-referred to the medical laboratory of Institut Pasteur du Cambodge. The time elapsed since hospital admission was not recorded for the samples originating from the hospitals; these samples therefore reflected both community-acquired and nosocomial infections.

Microbiological analysis

Each sample was seeded on specific media, as per the study laboratory procedures (Freney, 2007): chocolate agar, blood agar, and chromogen agar for urine samples (UriSelect 4 Medium); these were then incubated for 24–48 h at 37 °C in aerobic or aerobic and 5% CO_2 conditions. *Enterobacteriaceae* were identified using the API 20E gallery (bioMérieux, Marcy l'Etoile, France). Antimicrobial susceptibility testing (AST) was done by disk diffusion on agar technique, according to the recommendations issued by the

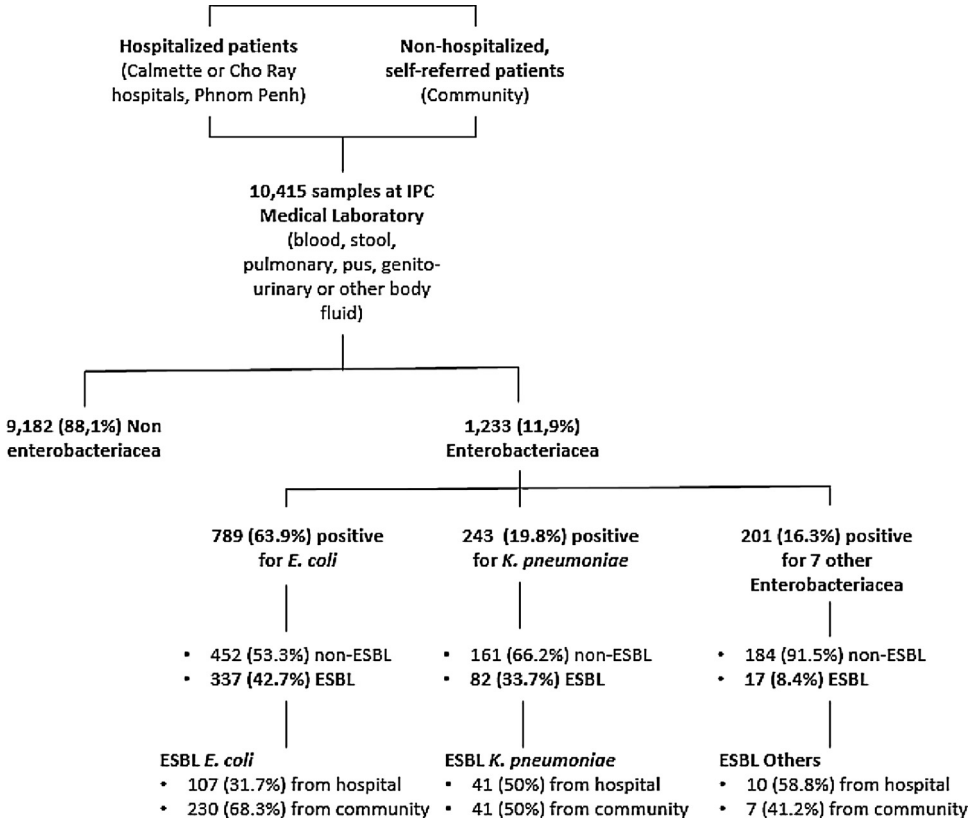

Figure 1. Sample origin and distribution, Medical Laboratory, Institut Pasteur du Cambodge, Cambodia 2012–2015.

Table 1
Distribution of ESBL-E isolates by anatomical site.

	ESBL/total species (%)				
	2012	2013	2014	2015	Total
Pulmonary	4/17 (23.5%)	5/18 (27.7%)	15/42 (35.7%)	27/61 (44.2%)	51/138 (36.9%)
Escherichia coli	0/4 (0.0%)	0/4 (0.0%)	6/11 (54.5%)	13/19 (68.4%)	19/38 (50%)
Klebsiella pneumoniae	4/10 (40%)	5/11 (45.4%)	8/25 (32.0%)	14/31 (45.1%)	31/77 (40.2%)
Other	0/3 (0.0%)	0/3 (0.0%)	1/6 (16.6%)	0/11 (0.0%)	1/23 (4.3%)
Blood culture	0/7 (0.0%)	2/5 (40.0%)	3/6 (50%)	2/9 (22.2%)	7/27 (25.9%)
Escherichia coli	0/2 (0.0%)	1/1 (100.0%)	1/1 (100.0%)	2/5 (40.0%)	4/9 (44.4%)
Klebsiella pneumoniae	0/1 (0.0%)	1/1 (100.0%)	2/3 (66.7%)	0/0 (0.0%)	3/5 (60.0%)
Other	0/4 (0.0%)	0/3 (0.0%)	0/2 (0.0%)	0/4 (0.0%)	0/13 (0.0%)
Urine	37/120 (30.8%)	80/194 (41.2%)	77/180 (42.7%)	80/192 (41.6%)	274/686 (39.9%)
Escherichia coli	31/94 (32.9%)	69/158 (43.6%)	73/163 (44.7%)	74/154 (48%)	247/569 (43.4%)
Klebsiella pneumoniae	3/11 (27.2%)	8/24 (33.3%)	3/10 (30%)	3/18 (16.6%)	17/63 (26.9%)
Other	3/15 (20.0%)	3/12 (25%)	1/7 (14.2%)	3/20 (15%)	10/54 (18.5%)
Genital	4/16 (25%)	7/30 (23.3%)	10/39 (25.6%)	11/44 (25%)	32/129 (24.8%)
Escherichia coli	2/12 (16.6%)	3/17 (17.6%)	7/21 (33.3%)	9/28 (32.1%)	21/78 (26.9%)
Klebsiella pneumoniae	2/4 (50.0%)	3/7 (42.8%)	3/10 (30.0%)	2/10 (20%)	10/31 (32.2%)
Other	0/0 (0.0%)	1/6 (16.6%)	0/8 (0.0%)	0/6 (0.0%)	1/20 (5%)
Stool	0/2 (0.0%)	0/3 (0.0%)	0/11 (0.0%)	0/7 (0.0%)	0/23 (0.0%)
Escherichia coli	Commensal flora, non-documented				
Klebsiella pneumoniae					
Other (Salmonella, Shigella)	0/2 (0.0%)	0/3 (0.0%)	0/11 (0.0%)	0/7 (0.0%)	0/23 (0.0%)
Pus	3/39 (7.6%)	6/25 (22.2%)	27/73 (36.9%)	36/93 (38.7%)	72/230 (31.3%)
Escherichia coli	2/9 (22.2%)	3/4 (75%)	16/33 (48.4%)	25/49 (51%)	46/95 (48.4%)
Klebsiella pneumoniae	1/9 (11.1%)	1/13 (7.6%)	10/21 (47.6%)	9/24 (37.5%)	21/67 (31.3%)
Other	0/21 (0.0%)	2/8 (25%)	1/19 (5.26%)	2/20 (10%)	5/68 (7.3%)
Total	48/201 (23.8%)	100/275 (36.3%)	132/351 (37.6%)	156/406 (38.4%)	436/1233 (35.3%)
Escherichia coli	35/121 (28.9%)	76/184 (41.3%)	103/229 (44.9%)	123/255 (48.2%)	337/789 (42.7%)
Klebsiella pneumoniae	10/35 (28.5%)	18/56 (32.1%)	26/69 (37.6%)	28/83 (33.7%)	82/243 (33.7%)
Other	3/56 (5.3%)	6/35 (17.1%)	3/53 (5.6%)	5/68 (7.3%)	17/201 (8.4%)

ESBL-E, extended-spectrum beta-lactamase *Enterobacteriaceae*.

Antibiogram Committee of the French Society for Microbiology/European Committee on Antimicrobial Susceptibility Testing (CA-SFM/EUCAST) (Microbiologie CdladlSFd, 2015). The disk synergy method is the most practical technique to detect resistance among ESBL-E. All AST were read automatically by the OSIRIS expert system (Bio-Rad, Marnes-la-Coquette, France) to determine the antibiotic susceptibility of the strains. The Etest method (bioMérieux) was used to determine minimum inhibitory concentration (MIC) titers for selected resistance phenotypes (according to CA-SFM/EUCAST) (Microbiologie CdladlSFd, 2015).

ESBL-E detection was performed as per the CA-SFM/EUCAST guidelines (Microbiologie CdladlSFd, 2015) with the disk synergy method: cefotaxime (5 μg), ceftazidime (10 μg), cefepime (30 μg), and aztreonam (30 μg) disks (Bio-Rad) were placed by an automatic disk dispenser on Mueller–Hinton agar at a center-to-center distance of 30 mm from a clavulanate disk (amoxicillin–clavulanic acid 20/10 μg or ticarcillin–clavulanic acid 75/10 μg). The presence of ESBL was inferred when the inhibition zone around any of the five antibiotic disks was enhanced on the side of the disk containing clavulanic acid, resulting in a characteristic champagne cork- or keyhole-shaped zone (Jarlier et al., 1988).

Statistical analysis

Data on sample type, origin, and results were entered into Excel (Microsoft, Redmond, WA, USA). Percentages were computed for each study year and by sample origin (community or hospital). Global percentages were compared using Fisher's exact test. The latter tests and Chi-square for trend analyses were performed using R software (R Foundation for Statistical Computing, Vienna, Austria; 2017).

Ethical considerations

All samples were collected through routine clinical activity. No socio-demographic factors were analyzed and the bacteriological results were anonymized and aggregated before analysis.

Results

A total of 10 415 samples were tested during the study period (Figure 1). Among the 1233 *Enterobacteriaceae* isolated, the most commonly isolated organisms were *Escherichia coli* (789; 63.9%) and *Klebsiella pneumoniae* (243; 19.8%) (Table 1). The species of bacteria identified differed significantly between the patients in the community and those in the hospital: *E. coli* was found mainly in community patients (chi-square for trend = 21.2, $p < 0.0001$) and *K. pneumoniae* in hospital patients (chi-square for trend = 8.2, $p < 0.0001$). Furthermore, urine contained more *E. coli* than the other samples (chi-square for trend = 248.56, $p < 0.0001$) and pulmonary samples contained more *K. pneumoniae* than the other samples (chi-square for trend = 176.25, $p < 0.0001$). The other *Enterobacteriaceae* were identified mostly in blood (chi-square for trend = 86.02, $p < 0.0001$).

Among the 1233 *Enterobacteriaceae* isolated, ESBL was found in 337/789 *E. coli* (42.7%) and 82/243 (33.7%) *K. pneumoniae*. Several other species of *Enterobacteriaceae* (belonging to seven genera: *Enterobacter spp*,*Proteus spp*, *Salmonella spp*, *Shigella spp*, *Citrobacter spp*, *Edwardsiella spp*, and *Providencia spp*) were isolated in 201 (1.9%) of the 10 415 samples. Among these, 17 (8.4%) were ESBL-producing (*Enterobacter cloacae*, *Enterobacter aerogenes*, *Morganella morganii*, and *Proteus mirabilis*). No ESBL-producing *Salmonella spp* or *Shigella spp* were found in this study.

Table 2
Frequency of ESBL-E-positive isolates in community- and hospital-acquired infections.[a]

Year	ESBL *E. coli* ($n = 337$)/*E. coli* ($n = 789$)		ESBL *K. pneumoniae* ($n = 82$)/*K. pneumoniae* ($n = 243$)		Other ESBL-E ($n = 17$)/Other *Enterobacteriaceae* ($n = 201$)		Total ESBL/Total species
	H $n = 234$	C $n = 555$	H $n = 106$	C $n = 137$	H $n = 88$	C $n = 113$	$n = 1233$
2012	4/19	31/102	4/14	6/21	2/18	1/27	48/201
2013	11/21	65/163	6/16	12/40	2/11	4/24	100/275
2014	40/86	63/143	15/37	11/32	2/36	1/17	132/351
2015	52/108	71/147	16/39	12/44	4/23	1/45	156/406
Total ESBL	107/234	230/555	41/106	41/137	10/88	7/113	436/1233

ESBL-E, extended-spectrum beta-lactamase *Enterobacteriaceae*.
[a] H: sample forwarded by a hospital; C: sample forwarded from the community.

Of the 436 ESBL-E isolates, 274 (63%) were obtained from urine, 72 (16.5%) from pus, 51 (12%) from pulmonary samples, and 32 (7.5%) from genital swabs; only seven (1.6%) were from blood cultures and 0% were from stool. No statistically significant difference in the distribution of ESBL-E isolates was noted by anatomical site (*E. coli* chi-square for trend = 9.02, $p = 0.06$; *K. pneumoniae* chi-square for trend = 4.04, $p = 0.40$).

The study results showed a progressively increasing frequency of ESBL-E: 23.8%, 36.3%, 37.6%, and 38.4% in 2012, 2013, 2014, and 2015, respectively (chi-square for trend = 11.6, $p < 0.001$) (Table 1). The proportion of ESBL-producing *E. coli* (ESBL-EC) showed a strong annual increase, rising from 28.9% in 2012 to 41.3% in 2013, 44.9% in 2014, and 48.2% in 2015 (chi-square for trend = 12.404, $p < 0.05$). These percentages were 28.5%, 37.6%, 42%, and 33.7%, respectively, for *K. pneumoniae* (chi-square for trend = 3.117, $p = 0.37$). Concerning ESBL-EC, this increase by year was not significantly associated with the origin of the sample: urine chi-square for trend = 5.64 ($p = 0.13$), genital chi-square for trend = 1.99 ($p = 0.57$), blood chi-square for trend = 4.14 ($p = 0.24$), pus chi-square for trend = 3.50 ($p = 0.32$); however a slight effect was seen for lung (chi-square for trend = 10.67, $p = 0.01$).

No statistically significant difference was recorded between the species of ESBL bacteria and patients from the hospital or the community: *E. coli* chi-square for trend = 0.67207, $p < 0.41$; *K. pneumoniae* chi-square for trend = 2.13, $p < 0.14$.

The frequency of ESBL-E was 63% in the samples from the community (276/436) and 37% (160/436) in the samples from the hospitals (Table 2). Among ESBL-EC, 45.7% (107/234) originated from hospitalized patients and 41.8% (230/550) from the community patients ($p = 0.41$). There was no significant difference in the percentages of ESBL-producing *K. pneumoniae* (ESBL-KP) between samples from hospital patients and samples from community patients: 38.6% (41/106) and 29.9% (41/137), respectively ($p = 0.14$).

The ESBL-producing *E. coli* and *K. pneumoniae* phenotypes were associated with varying resistance profiles, but they remained susceptible to ertapenem and imipenem in 100% of cases (Table 3).

Among ESBL-producing *E. coli* and *K. pneumoniae*, resistance to ciprofloxacin (indicating cross-resistance to all fluoroquinolones) was found in 82% and 72%, respectively. Resistance to co-trimoxazole was observed in 80% of ESBL-EC and 88% of ESBL-KP.

Regarding aminoglycosides, the majority of ESBL-EC and ESBL-KP remained sensitive to amikacin (46% for both). A small percentage of these were resistant to all aminoglycosides (6% for ESBL-EC and 15% for ESBL-KP). Furthermore, 11 ESBL-EC and five ESBL-KP were found to be resistant to all aminoglycosides except gentamicin.

Finally, some ESBL-EC and ESBL-KP were resistant to fluoroquinolones, co-trimoxazole, and aminoglycosides (phenotype 'KTGAN'), but remained susceptible to fosfomycin and carbapenems. This profile was found in 14 strains of ESBL-EC and 11 strains of ESBL-KP.

Discussion

In this study on samples collected in 2012–2015 and analyzed at the medical laboratory of the Institut Pasteur du Cambodge, a gradual and statistically significant increase in the proportion of ESBL-E was found. The most commonly identified strains were *E. coli* and *K. pneumoniae*, present in 77% and 19% of positive isolates, respectively. This reflects the emergence of ESBL-EC in the community in Cambodia, as described since the 2000s in other countries (Pitout et al., 2008).

ESBLs were first identified in *K. pneumoniae* and *Enterobacter spp*, commensal species usually found in low concentrations in the gastrointestinal tract, and particularly responsible for nosocomial infections (Brun-Buisson et al., 1987; Sirot et al., 1987). *E. coli*, a ubiquitous bacterium of the normal intestinal flora, can cause both nosocomial and community infections. The species of bacteria found in this study differed significantly between the patients from the community (*E. coli*) and the hospital (*K. pneumoniae*). This is probably due to the fact that *E. coli* is often responsible for benign lower urinary tract inflammation not requiring hospitalization, in contrast to pulmonary infections due to *K. pneumoniae*. This is confirmed by the higher prevalence of *E. coli* in urine and of *K. pneumoniae* in pulmonary samples.

Human transmission of ESBL-EC or ESBL genes is anticipated. This is especially true in developing countries, which complicates the control of dissemination. Transmission levels of *E. coli* have been correlated with the overall level of development of a country (Mody and O'Reilly, 2015). Considering the major clinical impact of ESBL-EC, widespread diffusion of these enzymes and phenotypes constitutes a new type of fecal peril and a major threat to global health (Zhao et al., 2015).

In the Asia-Pacific region, ESBL-E have been isolated from intra-abdominal pus, with 50.8% being ESBL-EC and 45.5% being ESBL-KP in Thailand and 34.4% being ESBL-EC and 39.1% being ESBL-KP in Vietnam (Hawser et al., 2009). The present authors conducted another study (unpublished) of stool samples collected over a 10-month period by the laboratory of the Institut Pasteur du Cambodge, which showed an ESBL proportion of 69% (165/241), including 91% ESBL-EC (150/165). A previous study, published study showed that the intestinal carriage of ESBL- producing *Enterobacteriaceae* was found for 72% in Laos and 51% in Vietnam, including 70% of ESBL-EC in Laos and 47% of ESBLE-EC in Vietnam, with 47% being *E. coli* (Nakayama et al., 2015). Similar rapid increases in ESBL-EC have also been found in many European studies (Fouquet et al., 2012; Nijssen et al., 2004).

The emergence of ESBL-E in developing countries and especially in the community is cause for serious local and international concern (Marchaim et al., 2010; Zahar et al., 2009). These bacteria are also resistant to other families of antibiotics, due to associated genes on the same plasmids or to chromosomal mutations (Chopra et al., 2003; Fierer et al., 1999). Resistance to gentamicin

Table 3
Resistance to fluoroquinolones, co-trimoxazole, and/or aminoglycosides in isolated strains.

	Resistance in *Escherichia coli*				Resistance in *Klebsiella pneumoniae*			
	ESBL-pos $n = 337$ (%)	ESBL-neg $n = 452$ (%)	Total $n = 789$ (%)	*p*-Value	ESBL-pos $n = 82$ (%)	ESBL-neg $n = 161$ (%)	Total $n = 243$ (%)	*p*-Value
Fluoroquinolones	275 (82%)	285 (63%)	560 (71%)	0.0048	59 (72%)	34 (21%)	71 (29%)	<0.0001
Co-trimoxazole	271 (80%)	309 (67%)	580 (73.5%)	0.06	72 (88%)	46 (29%)	118 (48.5%)	<0.0001
Sensitive to amikacin	155 (46%)	51 (11.30%)	206 (26%)	0.0003	38 (46%)	9 (5.60%)	47 (19.5%)	<0.0001
Resistance to all aminoglycosides	19 (6%)	10 (2.20%)	29 (3.70%)	0.13	12 (15%)	0	12 (5%)	<0.0001

ESBL, extended-spectrum beta-lactamase; pos, positive; neg, negative.

(phenotype G, GT, GTN, and KTGN) was higher than the resistance to all aminoglycosides (phenotype KTGAN): 46% and 6%, respectively, for ESBL-EC; 46% and 15%, respectively, for ESBL-KP. For *Enterobacteriaceae* not producing ESBL, the vast majority of strains remained susceptible to aminoglycosides. Resistance to aminoglycosides depends on the type of beta-lactamase produced (Almaghrabi et al., 2014; Schwaber et al., 2005).

The worrying levels of co-resistance to antibiotics are attributed to selection pressure due to the massive over-prescribing of broad-spectrum antibiotics (Laxminarayan et al., 2013). Antibiotics known to select antimicrobial-resistant strains are amoxicillin–clavulanic acid, cephalosporins (with a greater risk for third- and fourth-generation cephalosporins, by oral route), and especially fluoroquinolones. The indiscriminate use of fluoroquinolones has increased resistance to these molecules significantly (Paterson et al., 2000; Wang et al., 2004). Moreover, the use of fluoroquinolones has been identified first as a risk factor for colonization and infection with Gram-negative bacteria producing ESBL and second as a risk factor for Ambler class B carbapenemase acquisition (Coque et al., 2008). Carbapenems retain very good activity against ESBL-E. As no new antibiotics to treat ESBL-E infections are currently available, especially in developing countries, the rational use of carbapenems is a priority to avoid a therapeutic dead-end (Philippon, 2013).

The control of the spread of emerging highly resistant bacteria is based on a dual strategy of reducing the prescription of antibiotics to limit selection pressure and preventing spread from carriers (Janvier et al., 2011). In order to prevent cross-transmission through the dissemination of ESBL strains and resistance genes, strategies must combine hospital hygiene measures (prevention of cross-transmission and prevention of actual bacteria and their resistance genes spreading by controlling excreta) and measures for hygiene in the community. The prescription of third-generation cephalosporins and fluoroquinolones should be strictly and continuously supervised to prevent the emergence of multi-resistant bacteria selected by prescribed antibiotics. It is of course advisable to make 'better use' of antibiotics, prioritizing the use of molecules that exert the weakest selection pressure on ESBL-EC, but also to make 'least use' of antibiotics. This is a considerable challenge in Cambodia, a country where even fluoroquinolones are available over the counter, dispensed for a day or two at a time, and where physicians and pharmacists are known to misuse or overuse these antibiotics which are considered "much more effective" (Goyet et al., 2014). Antibiotic consumption must be limited in hospitals and in the community, and not only for human medicine, but also for animal health (Haenni et al., 2014).

The present study finding of an increase in antimicrobial resistance may be subject to bias and limitations. First, the high and rising prevalence of ESBL may be due to recruitment bias in the patient sample. The medical laboratory at Institut Pasteur du Cambodge is a reference center for microbiology in Phnom Penh. Microbiological analyses are performed for a fee and this may have selected hospitalized patients and comparatively wealthy walk-in patients. Both of these groups may be more prone to use antibiotics. In Cambodia, however, antibiotics such as quinolones are available over the counter. Furthermore, most other private and public sector microbiology laboratories are known to charge similar fees. The cost of analysis is, therefore, not likely to have resulted in the selection of a particular sample of patients for this study. Second, a bias in patient recruitment may have over-represented the co-resistance profiles. These patients may be more prone to repeat hospital admissions and therefore multiple exposures to various classes of antibiotics. Furthermore, samples may be taken after a first, probabilistic course of antibiotics has failed. The high co-resistance profile, however, may be explained by the high usage rates of fluoroquinolones for various indications (Paterson et al., 2000). Additionally, aminoglycosides have been used widely in Cambodia, a country with a high tuberculosis burden (Garneau-Tsodikova et al., 2016). Third, the choice of technique to correct the increase in antimicrobial resistance may have led to documentation bias. However, Institut Pasteur du Cambodge has consistently used a CA-SFM/EUCAST-recommended strategy since 2003. The results presented herein are therefore comparable to those from previous studies in Cambodia, as well as many other studies in the region and in Europe. Furthermore, the thoroughness of patient recruitment (hospital and ambulatory patients) and the methods used in the reference laboratory remained consistent across the 2012–2015 study period, and the results from this patient series can be extrapolated more generally to Cambodia.

Further studies could provide additional interesting information. Genetic characterization of the ESBL isolates was performed and the results should be published soon.

In conclusion, the high prevalence and spread of multidrug resistance in *Enterobacteriaceae* raises public health concerns due to the very real risk of a therapeutic dead-end. A comprehensive system to prevent the emergence and transmission of ESBL must be implemented across the board, especially in developing countries such as Cambodia. Surveillance systems must be implemented or maintained to assess the real-life impact of such measures on the ecology of ESBL-E. As a non-state actor officially engaged with the World Health Organization since its 138th session in January 2016 (document EB138/NGO/2), the International Network of Pasteur Institutes has and will continue to support antimicrobial resistance surveillance in the Mekong Region and beyond.

Conflict of interest

None.

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