Original Article

Isolation of Extended Spectrum ß-lactamase (ESBL) Producing Bacteria from Urban Surface Waters in Malaysia

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Abstract

***Background*: This was a preliminary study to test for the presence of multiple antibiotic-resistant extended spectrum β-lactamase (ESBL) producing bacteria in Malaysian urban surface waters. Although the literature review revealed several published papers on clinical ESBL isolates in Malaysia, none were found on ESBL isolates obtained from local surface waters.**

***Methods:* Isolated bacterial species were tested for resistance to cefotaxime, amoxicillin/clavulanate and aztreonam, and susceptibility to imipenem and meropenem using antibiotic susceptibility testing (AST) by disc diffusion. This served as a screening step to detect bacteria that could be potential ESBL species. 16S ribose ribonucleic acid (rRNA) polymerase chain reaction (PCR) testing with two clusters of *bla* (β-lactamase) gene primers was used to test for the *bla* genes CTX-M (Groups 1, 2, 9), OXA-1, SHV and TEM.**

***Results:* A total of 19 isolates were found, possessing at least one of the *bla* genes tested for. There was a relatively high occurrence of CTX-M genes (84.2%) among these, followed by TEM genes (47.4%). The isolates were identified as Enterobacteriaceae (89.5%), predominantly *Escherichia coli* and *Klebsiella pneumoniae*.**

***Conclusion:* There appears to be a high occurrence of ESBL-bacteria in local surface waters, among these being opportunistic pathogens. The persistence and spread of these species in the environment poses a threat to exposed human populations.**

Keywords: *extended spectrum β-lactamase (ESBL), bla (β-lactamase) gene, surface water, opportunistic pathogen, antibiotic resistance*

Introduction

Urban surface waters comprise rivers, streams, lakes, and wetlands that are exposed to the relatively high population densities of cities and towns. Urban rivers are therefore often exposed to physical, chemical, and biological contaminants from their proximal environment.

Antibiotic-resistant bacteria are among the more hazardous biological contaminants found in urban surface waters. There are several mechanisms by which different bacteria develop antibiotic resistance, the synthesis of β-lactamase enzymes being one of these. These enzymes hydrolyse the β-lactam ring structure of the antibiotic, thereby rendering it inactive. Extended spectrum β-lactamase (ESBL) enzymes are a branch of β-lactamases that are able to target a broader spectrum of antibiotics, including penicillins and their complexes (e.g. amoxicillin-clavulanate), monobactams (e.g. aztreonam), and up to third generation cephalosporins (e.g. cefotaxime). However, they often remain susceptible to cephamycins and carbapenems (e.g. imipenem, meropenem) (1).

There are several hundred types of β-lactamase enzymes, coded for by different types of *bla* (β-lactamase) genes. Their individual differences lie in the amino-acid structure of the protein molecules (2). These enzymes are categorised into families, based on genetically similar characteristics. Several of these genes are specifically ESBL genes, some of the more common among them being CTX-M, TEM, and SHV families (3). Each ESBL bacterium may possess genes for one or more of these enzymes. Phylogenetically similar *bla* genes with structural likeness are grouped together within these gene families and are often targeted for detection and identification using PCR. For instance, *bla* genes coding for the TEM-1 enzyme are found within group 1 of the TEM family.

The most common bacteria found to possess ESBL genes include *Escherichia coli, Klebsiella*

pneumoniae, Pseudomonas sp., *Shigella* sp. and *Salmonella* sp., certain strains of which are capable of causing disease. These bacteria are widespread in the environment. The emergence and propagation of new and existing ESBL strains in our surroundings thus constitute a continuing threat in a clinical context (4). Far-reaching research is therefore being carried out in many countries such as the USA (4), South Africa (5) and China (6) where ESBL strains have been isolated. The origin of these contaminants has been linked to surrounding factories, industries, farming and agriculture, and nosocomial and domestic waste.

Given that most urban surface waters in Malaysia are exposed to similar kinds of contaminant sources and surroundings, it is likely that local urban rivers may also be contaminated with antibiotic-resistant bacteria, including ESBL species. Although clinical and nosocomial infections linked to ESBL have been studied and reported (7-9), there appears to be no published data on ESBL isolates from Malaysian urban surface waters.

There is substantial evidence to support the idea of increased morbidity and mortality with ESBL outbreaks, particularly in the nosocomial environment (10). The fact that ESBLs are multi-drug resistant makes them a greater threat because most species are resistant to third generation cephalosporins (antibiotics initially thought to be resistant to β-lactamase hydrolysis). Some ESBL types, such as TEM and OXA, have also been shown to possess resistance genes against β-lactamase inhibitors (e.g. IRT genes), making such species more of a threat (11). Furthermore, since *bla* genes are usually found encoded on mobile vectors such as plasmids (CTX-M, SHV) (12) and transposons (TEM) (13), the transfer of resistance between bacteria is easily facilitated. This implies the easy spread of disease in the event of outbreaks. Infections associated with ESBLs vary from minor conditions such as urinary tract infections to more severe conditions such as pneumonia and bacteraemia (4). Human exposure to ESBL strains in rivers may therefore prove to be hazardous.

The purpose of this study was to carry out a preliminary investigation for the presence of ESBL species, which may be potentially pathogenic, in the urban surface waters of Malaysia. The study was conducted in the city of Petaling Jaya, within the state of Selangor. Being a modern, industrialised city with a population density of over 9,700/km^2 (14), this city could be considered an ideal representation of an 'urban' region for the purpose.

Materials and Methods

The four sampling sites selected within the city of Petaling Jaya were one site along Sungai Kayu Ara (3.112086N,461.596438E), two sites along Sungai Pencala and one site at a large drain running perpendicular to Jalan Lagoon Selatan (3.06454N,461.598756E). One of the two sites along Sungai Pencala (3.095823N,461.633946E) was exposed to a more industrialised environment. This area had many factories and companies such as motor showrooms, car rental companies, food manufacturers and a free trade zone. Drains and pipes from these industries open into the river. The other site along Sungai Pencala (3.120217N,461.627487E) was relatively less industrialised, although it did run close to the main road. Sungai Kayu Ara's sample site was in a more residential area, with condominiums and houses in the surrounding area. However, one of the banks was badly maintained and had domestic waste dumped along it. Sampling was carried out early in the day on different days, ensuring that there was no rain prior to sample collection.

Following collection of water samples, isolation of bacteria was carried out according to the protocol used by Lu (15). This involved filtering the samples through filter membranes and then culturing the membranes on nutrient agar. The medium was prepared without and with the antibiotics ampicillin (0.03 μg.L^{-1}) and cefotaxime (0.06 μg.L^{-1}). These antibiotics provided the initial screening for the isolation of ESBL species because they are both β-lactam antibiotics, the former being a penicillin and the latter a third generation cephalosporin. Medium without antibiotic was used to avoid eliminating species that may be susceptible to these antibiotics but resistant to others (15).

Isolated gram-negative species were subjected to antibiotic susceptibility testing (AST) to test for resistance to aztreonam (monobactam), amoxicillin/clavulanate (penicillin combination), and cefotaxime (third generation cephalosporin), as well as susceptibility to the carbapenems imipenem and meropenem. These antibiotics were selected because they cover several classes of β-lactam antibiotics, and were concurrent with the protocol used by Lu (15) for the detection of ESBL species. Apart from these, they were also tested for resistance to cefepime, which is a fourth generation cephalosporin.

Isolates exhibiting the desired susceptibility characteristics were identified using 16S rRNA PCR with the primers 27f and 1492r, listed in Table 1 (15).

In order to confirm the ESBL trait among these isolates, 16S rRNA PCR was carried out with primers specific for several commonly occurring *bla* genes. These included TEM, SHV, OXA and CTX-M types, all of which encode for different ESBL enzymes. Phylogenetically different genes were grouped separately within their gene type, e.g. CTX-M-1, CTX-M-3 and CTX-M-15 genes of group 1 of CTX-M-type genes. The genes tested for were divided into two multiplex clusters in order to obtain a clear banding pattern because some expected band sizes were within a short range of one another. The primers used are listed in Table 1. Agarose (2%) gel electrophoresis was carried out on the purified PCR products to compare band sizes.

Results

In total, 19 potential ESBL isolates were obtained from all four sample sites. These showed resistance to cefotaxime, aztreonam and amoxicillin-clavulanate (four of these showing intermediate resistance to amoxicillin-clavulanate), as well as susceptibility to imipenem and meropenem (Table 2).

Molecular testing detected the presence of *bla* genes pertaining to one or both clusters tested (Multiplex I and Multiplex II, Table 1) in the 19 isolates obtained. Among these, a majority of 84.2% of the isolates (i.e. 16 out of 19 isolates) were found to possess one or more CTX-M genes. TEM genes were detected in nine of the isolates (47.4%). These results reflected those of past research, where CTX-M and TEM genes have been found to be the predominant *bla* genes found in ESBL species (3,15). The majority of these potential ESBL isolates were identified as Enterobacteriaceae, mostly *Klebsiella* sp., *Escherichia* sp., and *Enterobacter* sp. Again, this is a similar observation to those of most studies involving ESBL isolates, where the most commonly occurring species are of the genera Escherichia and Klebsiella (15,21,22).

The results of the PCR and gel electrophoresis carried out is summarised in Table 3, showing

Table 1: Primers used to identify bacterial isolates (15), and to test for *bla* genes in isolates showing potential ESBL characteristic; primer names, PCR clusters and target genes (for *bla* genes), sequences, and product sizes have been specified (3)

Primers	PCR Cluster	Targeted β-lactamase gene(s)	Sequence (5'-3')	Product Size (bp)
27f 1492r	–	–	AGAGTTTGATCCTGGCTCAG GGTTACCTTGTTACGACTT	1500
MultiTSO-T_for MultiTSO-T_rev	Multiplex I TEM, SHV and OXA-1-like	TEM variants (including TEM-1, TEM-2)	CATTTCCGTGTCGCCCTTATTC CGTTCATCCATAGTTGCCTGAC	800
MultiTSO-S_for MultiTSO-S_rev		SHV variants (including SHV-1)	AGCCGCTTGAGCAAATTAAAC ATCCCGCAGATAAATCACCAC	713
MultiTSO-O_for MultiTSO-O_rev		OXA-1, OXA-4, OXA-30	GGCACCAGATTCAACTTTCAAG GACCCCAAGTTTCCTGTAAGTG	564
MultiCTXMGp1_for MultiCTXMGp1-2_rev	Multiplex II CTX-M group 1, group 2, group 9	Variants of CTX-M group 1 including CTX-M-1, CTX-M-3, CTX-M-15	TTAGGAARTGTGCCGCTGYA[a] CGATATCGTTGGTGGTRCCAT[a]	688
MultiCTXMGp2_for MultiCTXMGp1-2_rev		Variants of CTX-M group 2 including CTX-M-2	CGTTAACGGCACGATGAC CGATATCGTTGGTGGTRCCAT[a]	404
MultiCTXMGp9_for MultiCTXMGp9_rev		Variants of CTX-M group 9 including CTX-M-9 and CTX-M-14	TCAAGCCTGCCGATCTGGT TGATTCTCGCCGCTGAAG	561

[a] Y = T/C; R = A/G.

the genes detected and identified in each of the isolates. A classification of the ESBL isolates according to their identities is shown in Table 4.

Discussion

It is evident from Table 3 that potential ESBL species were detected at all four sites sampled. This is despite the fact that the sampling sites did not have a common source, except for the two sites along Sungai Pencala.

Molecular testing detected the presence of *bla* genes pertaining to one or both clusters tested for (Multiplex I and Multiplex II, Table 3) in the 19 isolates obtained, deeming them to be potential ESBL producers. It is worth noting that there may be other ESBL-producing bacteria that were not detected during this investigation, because there is a wide array of other *bla* genes that they may possess. These include IMP, GES and PER *bla* genes, among others (3), which were not tested for because testing was only carried out for some of the more common *bla* genes, following the protocol used by Dallenne (3). CTX-M and TEM *bla* genes are believed to be the most widespread among the human population, while the harbouring strains of SHV and OXA species are also known to cause infections in some cases (19).

The *bla*CTX-M genes (Multiplex II, Table 3) appear to have a high occurrence, with 16 out of the 19 isolates (84.2%) possessing at least one of the three types of CTX-M genes tested for. The *bla*TEM gene tested for also had a high occurrence, with nine out of the 19 isolates (47.4%) showing its presence. These findings are in accordance with previous literature (3,15) where *bla*CTX-M genes were found to be of highest occurrence in clinical isolates in studies conducted in France

Table 2: Antibiotic susceptibility testing (AST) results for potential ESBL isolates; numerical value next to antibiotic name indicates concentration in μg.mL^{-1}. Isolates were tested, by disc diffusion method, for resistance to cefotaxime, aztreonam, and amoxicillin clavulanate, and susceptibility to imipenem and meropenem, to screen for potential ESBL characteristic. In addition, susceptibility to cefepime, a fourth generation cephalosporin, was also tested

Isolate Name	Sampling Site	Susceptibility					
		CTX 50	ATM 30	IPM 10	MEM 10	AMC 30	FEP 30
A7	Drain running perpendicular to Jalan Lagoon Selatan	R	R	S	S	R	R
A20		R	R	S	S	R	R
A42		R	R	S	S	R	R
A51		R	R	S	S	R	S
A53		R	R	S	S	R	S
A58		R	R	S	S	R	I
C10	Sungai Pencala (industrial area)	R	R	S	S	R	R
C11		R	R	S	S	I	I
C20		R	R	S	S	R	I
C28		R	R	S	S	I	S
C29		R	R	S	S	I	S
E7	Sungai Pencala (residential area)	R	R	S	S	R	I
E17		R	R	S	S	R	R
E18		R	R	S	S	R	R
E20		R	R	S	S	I	R
E22		R	R	S	S	R	R
Da9	Sungai Kayu Ara	R	R	S	S	R	S
Da20		R	R	S	S	R	S
Da21		R	R	S	S	R	S

Abbreviation: R = Resistant, I = Intermediate, S = Susceptible, x = CLSI limits not available to determine susceptibility, CTX – cefotaxime, ATM – aztreonam, IMP – imipenem, MEM – meropenem, AMC – amoxicillin/clavulanate, FEP – cefepime.

and in a Chinese urban river (respectively). Similarly, *bla*TEM genes were of note-worthy high occurrence too. Despite the potential hazardous consequences of having ESBL bacteria in surface waters, there appear to be no published data on ESBL bacteria in the surface waters of Malaysia. Hence, it is not possible to make a comparison with similar studies within the country.

However, in terms of clinical isolates, a study carried out by Subramaniam (20) resulted in the isolation of 11 *Escherichia coli (E. coli)* isolates harbouring the *bla*SHV gene. In a more recent study, Lim (9) has recorded an occurrence of 80% *bla*TEM, 20% *bla*CTX-M, 8% *bla*SHV and 5%

Table 3: Summary of *bla* genes detected in the 19 ESBL isolates tested. CTX-M genes were found to be the most commonly occurring among the tested isolates, while TEM genes showed the second highest occurrence. The detection of the tested genes in the isolates confirms the presence of potential ESBL species in local urban rivers

Isolate	BLAST Identity (from 16S rRNA PCR)	Sampling Site	E-value[a]	Max. ID	*bla* Multiplex I			*bla* Multiplex II (CTX-M)		
					TEM (inc 1,2) [800 bp]	SHV (inc 1) [713 bp]	OXA-1, 4, 30 [564 bp]	Group 1 (inc 1,3,15) [688 bp]	Group 2 (inc 2) [404 bp]	Group 9 (inc 9, 14) [561 bp]
A7	*Klebsiella pneumoniae*	Drain running perpendicular to Jalan Lagoon Selatan	0.0	100%	–	+	+	+	–	–
A20	*Klebsiella* sp.		0.0	99%	–	+	+	+	–	–
A42	*Klebsiella pneumoniae*		9e-175	100%	–	+	+	+	–	–
A51	*Chromobacterium violaceum*		0.0	100%	–	–	–	–	+	–
A53	*Escherichia coli*		8e-72	100%	+	–	–	–	–	–
A58	*Presumptive coliformb*		3e-107	100%	+	+	–	–	+	–
C10	*Escherichia coli*	Sungai Pencala (industrial area)	0.0	100%	+	–	–	–	–	+
C11	*Klebsiella pneumoniae*		0.0	99%	+	+	–	+	–	–
C20	*Chromobacterium* sp.		3e-179	100%	–	–	–	+	+	–
C28	*Presumptive coliformb*		0.0	100%	+	–	+	–	–	–
C29	*Presumptive coliformb*		5e-162	100%	+	–	+	–	–	–
E7	*Klebsiella pneumonia*	Sungai Pencala (residential area)	0.0	100%	–	+	+	+	–	–
E17	*Enterobacter* sp.		0.0	100%	+	–	–	–	+	–
E18	*Shigella* sp.		0.0	100%	–	–	+	+	–	–
E20	*Escherichia* coli		9e-134	100%						
E22	*Escherichia* sp.		9e-134	100%	+	–	–	+	–	–
Da9	*Enterobacter* sp.	Sungai Kayu Ara	0.0	100%	–	–	–	–	+	+
Da20	*Enterobacter* sp.		0.0	100%	–	–	–	–	+	+
Da21	*Enterobacter* sp.		1e-178	100%	–	–	–	–	+	+
	Percentage occurrence				47.4	31.6	36.8	42.1	42.1	21.1
									84.2	

Abbreviation: + = Specified *bla* gene present, - = No *bla* genes detected.
[a] E-value → background noise for a given match, i.e. likelihood of getting more hits for a blast.
[b] As per isolation medium, gram staining and microscopy.

*bla*OXA genes in ESBL positive *E. coli* isolates from Malaysian hospitals. The comparatively low occurrence of CTX-M genes in the study has been attributed to these types of genes originating in the less common environmental genus *Kluyvera* (9). The detection of significant amounts of CTX-M isolates in surface waters in the current study could mean that these numbers may rise over time, thereby posing a greater threat to health. The same is applicable for the other ESBL genes detected too.

PCR sequencing showed that the 19 isolates obtained were predominantly of the family Enterobacteriaceae (17/19 isolates, i.e. 89.5%). Among these, *Klebsiella* (26.3%), *Escherichia* and *Enterobacter* (each 21.1%) species appeared to occur most frequently (Table 4). This is in keeping with previous studies on ESBL, where most research and findings have been concentrated on Enterobacteriaceae. For instance, out of 56 ESBL isolates from an urban river in China, Lu (15) found 39 (69.6%) of these to be Enterobacteriaceae. Similar results have been obtained in other environments as well, such as in farming environments (21) and clinical settings (22), where *Klebsiella pneumoniae* and *Escherichia coli* were found to be the dominant ESBL species present. Given that most species of the genus *Enterobacter* include commonly found enteric bacteria, it is possible that the source of these bacteria might be faecal contamination from domestic sewerage and animal waste.

Based on the literature reviewed, ESBL production is mostly found to occur among enteric species. However this study has shown an occurrence of the ESBL trait among likely environmental bacteria too, in the form of two *Chromobacterium* sp. isolates obtained from the drain running perpendicular to Jalan Lagoon Selatan and from the industrial site along Sungai Pencala. This could be a result of contamination from the environment surrounding the sampled rivers, or of exposure of these bacteria to other (faecal) contaminants, from which gene transfer may have taken place. An unkempt and excessive plant growth was evident all along the banks of these sample sites. The garbage dump of mostly domestic waste that lay adjacent to the Sungai Pencala site may also be a contributory factor because it would increase contamination of the water as well as the surrounding environment. The said isolations may thus be an indication of an increasing presence and proliferation of the ESBL characteristic among environmental bacteria.

As ESBL species are usually resistant to most β-lactam antibiotics, few options are left for the treatment of ESBL-associated infections. Carbapenems and fourth generation cephalosporins are two of the best available options (4) but the AST results of this study (Table 2) show resistance to cefepime, a fourth generation cephalosporin, in eight out of the 19 potential ESBL isolates. This is reflective of some recent studies that show an increasing emergence of resistance to fourth generation cephalosporins (16,17). Paterson (18) has linked this resistance to hydrolysis by blaCTX-M gene coded β-lactamase enzyme. The current study shows some consistency with this observation; all nine isolates showing resistance to cefepime (Table 2) express at least one type of CTX-M gene (Tables 3). Although cefepime is usually effective against these bacteria and is known to cause few mild side effects (4), the emergence of such resistant species in the environment limits options for optimal treatment of ESBL infections, thereby reducing the recovery rate of ESBL patients.

In terms of locations, all four sampling sites have a somewhat consistent occurrence of ESBL species (Table 3), depicting the presence

Table 4: Summary of the types of bacteria present among the 19 ESBL isolates, showing percentage occurrence of each genus, their family and phylum. The potential ESBL isolates detected were identified to be predominantly *Klebsiella* sp., *Escherichia* sp., and *Enterobacter* sp., all of which belong to the family Enterobacteriacaea

Genus	No of isolates (%)	Family	Phylum
Klebsiella sp.	5 (26.3)	Enterobacteriaceae	Proteobacteria
Escherichia sp.	4 (21.1)		
Enterobacter sp.	4 (21.1)		
Presumptive coliform	3 (15.8)		
Shigella sp.	1 (5.3)		
Chromobacterium sp.	2 (10.5)	Neisseriaceae	

of the ESBL trait in the environment. The threat of this observation is amplified because the isolated species are known to possess pathogenic strains. Hence, the adverse impact of any sort of outbreak arising from pathogenic ESBL strains on the health, economy, and social well-being of the human population would be much greater than otherwise. The potential threat is worse because the water samples were collected from urban areas, where human populations are more concentrated. Any underdeveloped settlements lining these rivers would be drastically affected because hygiene and sanitation are usually below par in such settings. Humans may also be exposed to CTX-M, TEM, and SHV carrying pathogenic species in food and food products by the use of contaminated surface waters for farming and agriculture (23). The dynamic nature of city life would enhance the rapid spread of disease and outbreaks (24). The fact that ESBL strains possess multi-drug resistance deepens the gravity of the situation.

However, the possibility of some of the detected TEM and SHV genes being non-ESBL variants should also be mentioned here. For instance, it has been found in some research that non-ESBL TEM-1 genes may co-exist with CTX-M genes on the same plasmid, and may therefore be transmitted and detected (25). In the case of TEM genes being detected in the absence of CTX-M genes (such as isolates A53, C28 and C29 in Table 3), there is also a possibility of their corresponding resistance being detected as a result of the over-expression of these genes, even if they are not ESBL genes per se (23, 25). Nonetheless, further testing would be required to confirm such characteristics within the context of this study.

In terms of danger to human health, previous research highlight that potential ESBL species such as *K. pneumonia* and *E. coli* have a high tendency to possess and transfer *bla* genes (13). Transfer may occur by conjugation because the genes are often found on mobile elements like transposons and integrons (26). Some of these species may be pathogenic strains that have the potential to cause life-threatening diseases and widespread outbreaks. For instance, *bla*CTX-M and *bla*TEM genes in opportunistically pathogenic *Klebsiella* spp. have been associated with nosocomial infections and outbreaks of diarrhoea (6). Examples of outbreaks include the neonatal intensive care unit (NICU) ESBL E. coli outbreak at Luton & Dunston Hospital in England (27) and an outbreak of ESBL *Klebsiella pneumoniae* in an NICU in Southern India (28). Pathogenic strains of ESBL E. coli have been associated with nosocomial urinary tract infections (13).

ESBL *Salmonella* has recently been causing increasing health problems, such as elevated paediatric infections in South Africa (5). Several TEM gene isolates from *Salmonella* sp. have also been obtained from infants in hospitals in Spain (26). Although no ESBL *Salmonella* sp. were isolated in the current study, it should be noted that this survey involved a limited selection of surface waters in a single state of the country. There could in fact be a wide range of other ESBL species in other surface waters across the country. In addition, since *Salmonella* sp. usually require pre-enrichment in selective broth for optimal growth (13) (not carried out in this study), it is possible that *Salmonella* isolates may subsequently not have been detected.

Lehner (29) highlights the socio-economic effects associated with multi-drug resistant pathogens like ESBL-producing species. Apart from the higher risk of outbreaks, particularly nosocomial, they pose a mounting challenge to clinicians treating infected patients, scientists researching and developing antimicrobials and governments and hospitals bearing the rising costs of addressing these problems. It is important to raise public and health worker awareness in terms of prevention of such circumstances. This also underlines the need for governing authorities and health organisations to initiate measures to effectively control the release of contaminants into the environment. The lack of data on the prevalence of ESBL bacteria in local surface waters is reflective of the lack of sufficient studies on Malaysian surface waters in general, from a microbiological perspective.

As an extension to the study carried out, it would be of use to increase the number of sampling sites beyond the state of Selangor and across the country. This would be more representative of the overall bacterial populations and ESBL species in local surface waters. It would also be interesting to carry out further molecular testing for the presence of other *bla* genes, and to characterise and determine whether these genes are present on transferable plasmids, transposons or integrons, which would facilitate easy spreading.

Conclusion

It is evident from this study that local urban surface waters are contaminated with potentially pathogenic ESBL-producing bacteria, possibly

originating from domestic and hospital waste, farming and agriculture. The presence of ESBL in the environment poses potential risks of outbreaks of infection in the event of human exposure and highlights the need to be prepared for such circumstances in terms of funding and healthcare. In addition, the results show the importance of effectively controlling the release of contaminants into local surface waters. The findings of this preliminary study may form the basis for future work on Malaysian surface waters.

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Conflict of interest

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Authors' Contributions

Analysis and interpretation of the data, drafting of the article and final approval of the article: ST
Conception and design, critical revision of the article for the important intellectual content, final approval of the article and obtaining of funding: LSM

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