Detection of Plasmid-Borne *β*-Lactamase Genes in Extended-Spectrum *β*-Lactamase (ESBL) and Non-ESBL-Producing *Escherichia coli* Clinical Isolates

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Increasing frequency of infections caused by antibiotic resistant *Escherichia coli* strains producing extended-spectrum β-lactamase (ESBL) needs to be addressed by continuous surveillance and accurate detection of specific ESBLs genes for more effective treatment. A total of 71 β-lactam drug resistant isolates (26 phenotypically ESBL-producing and 45 non-ESBL-producing) were observed to carry approximately 23 kb plasmids. These isolates were subjected to β-lactamase gene-targeted PCR to detect plasmid-encoded bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M\ group1}$ and $bla_{CTX\text{-}M\ group9}$ genes. BLAST analysis of amplicons revealed that plasmid-encoded bla_{TEM} is most prevalent in both ESBL and non-ESBL-producing *E. coli* isolates. Plasmid-encoded bla_{SHV} gene was only detected in 8 non-ESBL-producing isolates and explanation of such observation awaits additional studies to detect the possibility that the gene could be in the chromosomal DNA or to test the prevalence of the plasmid-encoded gene with more isolates. Twelve isolates of the ESBL-type $bla_{CTX\text{-}M}$ were identified from phenotypically identified ESBLs, comparable with 13 isolates detected with bla_{TEM}. This observation suggests that the relatively newly emerging ESBL-type CTX-M is continuously increasing as one of the new β-lactamase derivatives among ESBL-producing *E. coli* in the clinical setting. This study reveals that there is discrepancy between the results of the phenotypic observation and genotypic analysis showing that the presence of ESBL-associated β-lactamase genes may be undetected when using the conventional phenotypic approach. Mutation in these unexpressed genes may result to ESBL antibiotic resistance, suggesting that the unexpressed and undetected genes may serve as reservoir for ESBL genes.

Key words: CTX-M, ESBL, *Escherichia coli*, SHV, TEM

INTRODUCTION

Drug resistance among bacteria is largely attributed to their production of β-lactamase enzyme that can hydrolyze or inactivate the β-lactam drugs that interfere with bacterial cell wall synthesis (Bauman 2004). β-lactam drugs include the antibiotics penicillins, cephalosporins, cephamycins, carbapenems, mono-bactams and β-lactamase inhibitors. Infections caused by members of *Enterobacteriaceae* such as *Escherichia coli,* are most often difficult to treat due to their resistance particularly to β-lactam drugs.

Innovations in drug technology introduced new derivatives of β-lactam drugs that are more potent, broader in spectrum of target organisms and more resistant to the hydrolytic activity of β-lactamase enzyme. When a new

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β-lactam drug derivative is developed, new β-lactamase derivatives usually emerge, as a result of the selective pressure due to the use and abuse of the β-lactam drug. Because of the increased spectrum of bacterial activity against new expanded-spectrum β-lactams, particularly the cephalosporins, the β-lactamases are called extended-spectrum β-lactamases or ESBLs (Paterson and Bonomo 2005). In general, ESBLs are β-lactamases that are capable of conferring resistance to penicillins, first-, second- and third-generation cephalosporins, by hydrolyzing these antibiotics. ESBLs, however, are inhibited by   β-lactamase inhibitors such as clavulanic acid (Paterson and Bonomo 2005).

The rise in number of antibiotic resistant pathogens were shown in some antimicrobial surveillance data conducted in the Philippines. The prevalence of ESBL-producing *Enterobacteriaceae* was reported at the Makati Medical Center (Villanueva et al. 2003) and the Philippine General Hospital (Bomasang and Mendoza 2003). A similar observation was reported by Lucena et al. (2012) at Mindanao Sanitarium and Hospital in Southern Philippines, which showed an increase in resistance of Gram-negative from 1.69% in 2005 to 7.38% in 2008**.** In a retrospective study on ESBL-producing bacteria in the Philippines from 1999-2013, the prevalence of ESBL-producing *K. pneumoniae* and *E. coli* ranged from 10-43.24% and 4-20.9%, respectively (Lota and Latorre 2014).

Different groups of the β-lactamase gene (*bla*) are found either in chromosomal DNA or plasmid (Schmitt et al. 2007). The presence of the gene in plasmids further facilitated its transfer to different species of bacteria. The TEM and SHV β-lactamases were the first plasmid-mediated enzymes, from which many of the ESBLs have been derived (Bush and Jacoby 2010). A few point mutations at selected loci within the gene encoding these enzymes gave rise to the extended-spectrum phenotype, which now accounts for many ESBLs identified. TEM and SHV-derived ESBLs can hydrolyze the third generation cephalosporins particularly ceftazidime. At the beginning of the 21st century, a new family of plasmid-mediated ESBLs called CTX-M, which preferentially hydrolyze cefotaxime, became predominant in European countries, and started to spread in Southeast Asia including the Philippines.

Monitoring of ESBLs should not be limited to phenotypic screening since there had been reports showing the discrepancy in the phenotypic and genotypic detection (Yazdi et al. 2012; Xu et al. 2014). This study is focused on the isolation of plasmids from ESBL-producing and non-ESBL-producing *E. coli* clinical isolates and PCR detection of plasmid-borne β-lactamase genes (*bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M \ group1}$ and *bla*$_{CTX-M \ group9}$) that confer antibiotic resistance phenotype. The relative distribution of each gene among the *E. coli* isolates studied was analyzed and presence of unexpressed ESBL-associated genes in phenotypically non-ESBL isolates was evaluated. The presence of unexpressed ESBL genes may confer ESBL phenotype to pathogens after acquiring the needed mutation. It is therefore necessary to consider unexpressed ESBL genes in making appropriate steps to address problems associated with the increasing frequency and rapid spread of ESBL-producing bacteria. Consequently, the new information can be useful in developing accurate protocols for detecting ESBLs and treating infections.

METHODS

Escherichia coli Clinical Isolates
A total of 72 β-lactam resistant clinical *E. coli* isolates from selected hospitals in Luzon, Philippines (Cruz et al. 2014) were subjected to plasmid isolation procedure. Seventy-one out of 72 isolates that were positive for plasmid included 45 isolates classified as phenotypically non-ESBL and 26 isolates classified under phenotypically ESBL based on results of a previous study (Cruz et al. 2014) using the Double-disk Synergy Test (DDST).

Plasmid Extraction
Escherichia coli isolates were sub-cultured in Luria-Bertani broth and incubated for 24 hours prior to extraction. Plasmid was extracted from each isolate using commercially available plasmid extraction kit (Purelink Quick Plasmid MiniPrep by Invitrogen, USA). Extracted plasmids were subjected to agarose (0.8%) gel electrophoresis and visualized under UV (Alpha Innotech,USA) after staining with ethidium bromide and photographed by Alpha DigiDoc Pro (Alpha Innotech,USA).

Amplification of β-Lactamase Genes (*bla*)
Primers designed for detecting TEM-, SHV- and CTX-M (groups 1 and 9) β-lactamase-encoding genes (Dallenne et al. 2010, Figure 1), plasmid DNA templates from *E. coli* clinical isolates and available positive (TEM-1-producing *E. coli* ATCC 35218; CTX-M and SHV-producing clinical isolates confirmed by sequencing) and negative control (non-β-lactamase-producing *E. coli* ATCC 25922) isolates, were used in PCR. PCR was performed in a total volume of 25 μL PCR reaction containing 0.4 μM of each of pair of primers (Invitrogen, USA), 1 μL of DNA template, 0.4 mM dNTPs, 1.0-1.25 mM MgCl$_2$, 1 U of *Pfx* polymerase (Invitrogen, USA) and 5 uL of 10X amplification buffer.

The conditions for PCR included initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 15 sec, annealing at 59, 57, 61, and 58°C (for primers
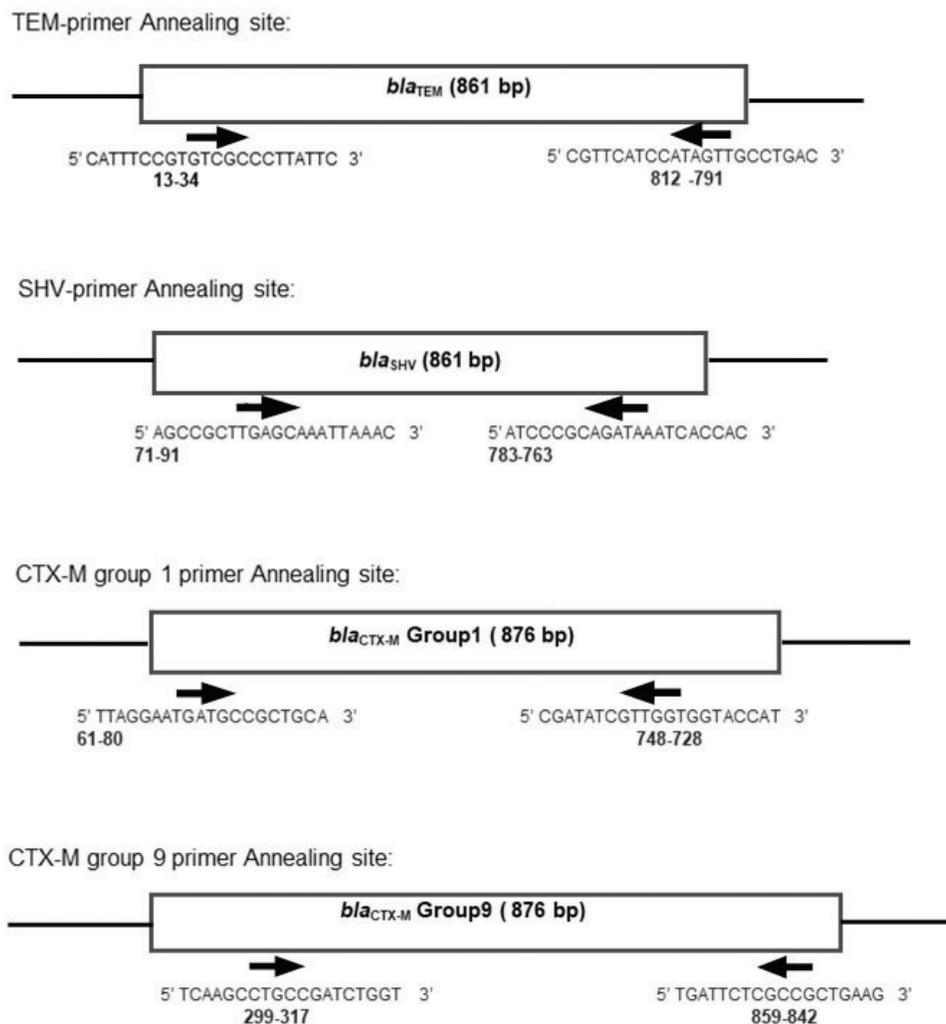
TEM-primer Annealing site:

bla_{TEM} (861 bp)

5' CATTTCCGTGTCGCCCTTATTC 3'
13-34

5' CGTTCATCCATAGTTGCCTGAC 3'
812 -791

SHV-primer Annealing site:

bla_{SHV} (861 bp)

5' AGCCGCTTGAGCAAATTAAAC 3'
71-91

5' ATCCCGCAGATAAATCACCAC 3'
783-763

CTX-M group 1 primer Annealing site:

bla_{CTX-M} Group1 (876 bp)

5' TTAGGAATGATGCCGCTGCA 3'
61-80

5' CGATATCGTTGGTGGTACCAT 3'
748-728

CTX-M group 9 primer Annealing site:

bla_{CTX-M} Group9 (876 bp)

5' TCAAGCCTGCCGATCTGGT 3'
299-317

5' TGATTCTCGCCGCTGAAG 3'
859-842

Figure 1. Annealing sites for primer sets targeting the major types of β-lactamase gene. The expected sizes of amplified internal gene fragments are 800 bp for bla_{TEM}, 713 bp for bla_{SHV}, 688 bp for $bla_{CTX-M\ grp1}$ and 561 bp for $bla_{CTX-M\ grp9}$.

targeting $bla_{TEM,}$ bla_{SHV}, $bla_{CTX-M\ group1}$, and $bla_{CTX-M\ group9}$, respectively) for 30 sec and extension at 68°C for 1 min, and final extension at 68°C for 4 min. Amplicons were visualized and photographed after agarose (1%) gel electrophoresis and ethidium bromide staining using Alpha DigiDoc Pro (Alpha Innotech, USA).

Identification and Distribution of *bla* Genes
Amplicons were sent for sequencing to 1st BASE (BASE Life Sciences Holdings, Singapore). Sequence data were compared to reference sequences available in the database using BLAST (http://www.ncbi.nlm.nih.gov/BLAST) sequence alignment software to identify the specific *bla* gene detected. Presence of one or more *bla* genes in each isolate was evaluated.

RESULTS

Plasmid-Borne β-Lactamase Genes (*bla*)
Plasmid profiles revealed that 71 isolates have one or more plasmids with bands representing amplicons ranging from 2 to 23 kb. A large band that is approximately 23 kb, was found common to all 71 isolates (Figure 2). Table 1 presents the number of ESBL and non-ESBL isolates that elicited expected amplicons after β-lactamase gene-targeted PCR using extracted plasmids from each isolate as template and primers that target four types of β-lactamase genes including the plasmid-mediated β-lactamase TEM-1, plasmid-encoded SHV-1, and two groups under CTX-M (CTX-M group 1 and CTX-M group 9; Bush and Jacoby 2010). Amplicons of about 800 bp, 688 bp and 561 bp that were expected for amplified
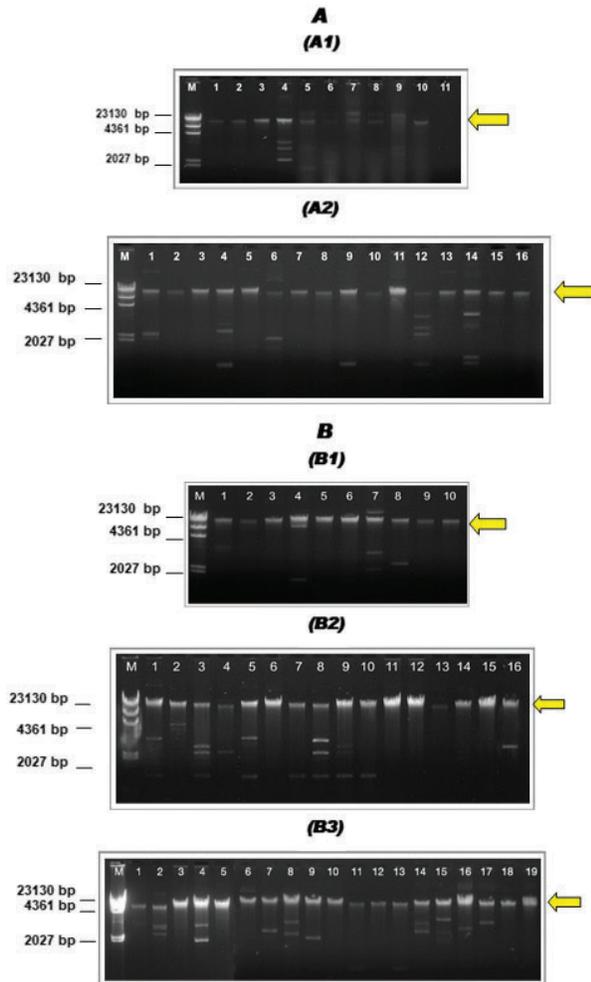
Figure 2. Plasmid profile of 27 ESBL-producing (A) and 45 non-ESBL-producing (B) *E. coli* clinical isolates. M is the high Molecular Weight marker II. Yellow arrow points to bands common to all isolates except the isolate in A1 lane 11.

Table 1. Results of β-lactamase gene-targeted PCR detection and identification.

bla gene detected	Number of isolates positive for expected size amplicon		Number of isolates positive for gene based on sequence analysis	
	ESBL	**Non-ESBL**	**ESBL**	**Non-ESBL**
TEM (~800 bp)	13	27	13	27
SHV (~713 bp)	0	8	0	8
CTX-M group 1 (~688 bp)	10	2	10	1
CTX-M group 9 (~561 bp)	8	18	2	0
No *bla* detected	7	17	NA	NA

β-lactamase genes for TEM (bla_{TEM}), CTX-M group 1 ($bla_{CTX-M\ group1}$), and CTX-M group 9 ($bla_{CTX-M\ group9}$), respectively, were observed (Figures 3 and 4).

Detection of β-Lactamase Genes (*bla*)

β-lactamase gene-targeted PCR primers (Figure 1) were used to amplify and detect *bla* which encode the four types of β-lactamase under Class A (Bush and Jacoby 2010), including the plasmid-mediated β-lactamase TEM-1, plasmid-encoded SHV-1, and two groups under CTX-M (CTX-M group 1 and CTX-M group 9). The number of ESBL-producing and non ESBL-producing isolates that exhibited expected amplification products of PCR using each type of β-lactamase gene-targeted primers are summarized in Table 1.
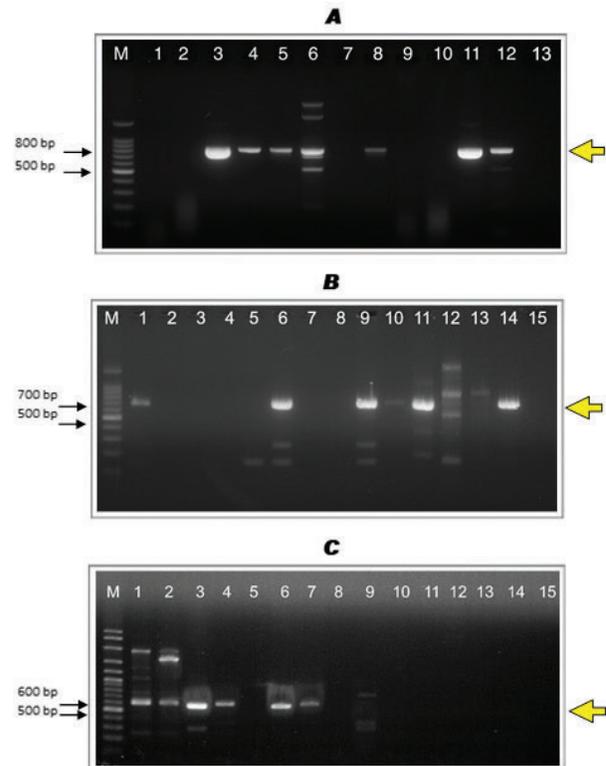

Figure 3. Representative amplicons of PCR using primers that target β-lactamase genes and plasmid DNA template from phenotypically ESBL-producing *E. coli* isolates. (**A**) Expected size amplicons (~800bp) of PCR using primers that target bla_{TEM} gene are in lanes 3-6, 8 and 11. Lanes 12 and 13 are results of PCR using templates from bla_{TEM}-positive control (ATCC 35218) and a negative control, respectively. (**B**) Expected size amplicons (~688bp) of PCR using primers that target $bla_{CTX-M\ group1}$ genes are in lanes 1,6,9,11 and 14. Lane 15 is the negative control. (**C**) Expected size amplicons (~561bp) of PCR using primers for $bla_{CTX-M\ group9}$ genes are in lanes 1-4, 6 and 7. Lane 15 is the negative control. **M** is the 100-bp ladder in A and B, and 100 bp Plus DNA ladder in C. Yellow arrows point to positions of expected size of β-lactamase gene amplicons.
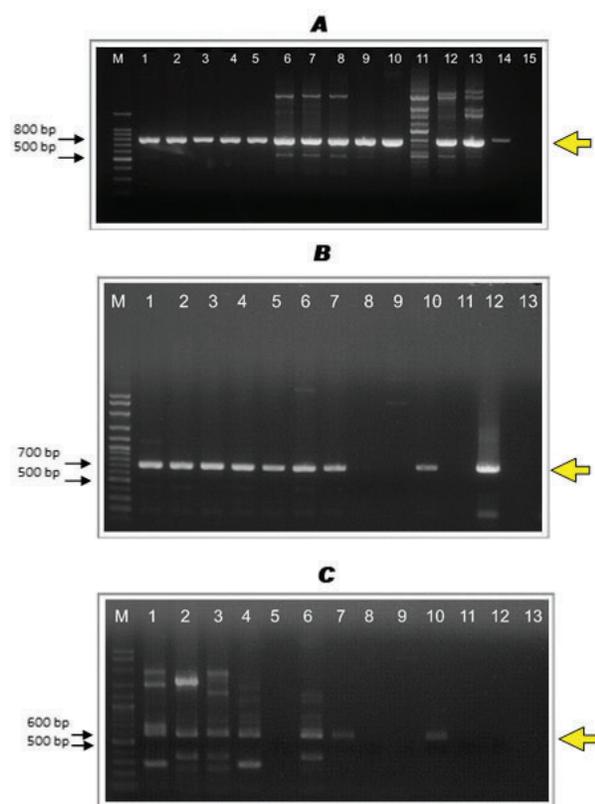
Figure 4. Representative amplicons of PCR using primers that target β-lactamase genes and plasmid DNA template from phenotypically non-ESBL-producing *E. coli* isolates. **(A)** Expected size amplicons (~800bp) of PCR using primers that target bla_{TEM} gene are in lanes 2-10 and 12-14. Lanes 1 and 15 are results of PCR using templates from bla_{TEM}-positive control (ATCC 35218) and a negative control, respectively. **(B)** Expected size amplicons (~713bp) of PCR using primers that target 713-bp for bla_{SHV} gene are in lanes 1-7 and lane 10. Lanes 12 and 13 are the positive and the negative control, respectively. **(C)** Expected size amplicons (~561bp) of PCR using primers for $bla_{CTX-M\ group9}$ genes are in lanes 1-4, 6,7 and 10 Lane 15 is the negative control. **M** is the 100-bp ladder in A and B, and 100 bp Plus DNA ladder in C. Yellow arrows point to positions of expected size of β-lactamase gene amplicons.

Amplicons of about 800 bp, 688 bp and 561 bp that were expected for amplified β-lactamase genes for TEM (bla_{TEM}), CTX-M group 1 ($bla_{CTX-M\ group1}$), and CTX-M group 9 ($bla_{CTX-M\ group9}$), respectively, were observed in both phenotypically ESBL-producing and non-ESBL-producing isolates (Figures 3 and 4). Expected 713-bp amplicon for SHV β-lactamase gene (bla_{SHV}) was not produced in ESBL-producing isolates but were observed in eight non-ESBL-producers (Figure 4).

BLAST Analysis of *bla* Gene Amplicons
BLAST analysis revealed that the target gene was detected in all bla_{TEM}-gene-targeted PCR for both ESBL and non-

ESBL-producing isolates (Table 1). Out of 13 amplicons from bla_{TEM}-gene-targeted PCR of ESBL-producing isolates, 7 exhibited 100% while 6 exhibited 99% sequence similarity with TEM-1 type (bla_{TEM-1}) gene. All the 27 amplicons of the bla_{TEM}-gene-targeted PCR in non-ESBL-producing isolates were also positive for bla_{TEM} gene with 17 of 27 exhibiting 100% and 10 of 27 exhibiting 99% sequence similarity with reported bla_{TEM-1} gene.

The bla_{SHV}-gene-targeted PCR using plasmid DNA templates from ESBL-producing isolates did not result in the amplification of expected 713 bp product (Table 1). Expected target gene was only amplified in bla_{SHV}-gene-targeted PCR in 8 non- ESBL-producing isolates. BLAST results showed that three of these eight isolates exhibit 100% sequence similarity of amplicons with bla_{SHV-1} gene, and the remaining five of eight, showed 96-99% similarity with SHV gene previously reported in ESBL-producing isolates. Nine of 10 amplicons from PCR using primers that target $bla_{CTX-M\ group\ 1}$ gene in ESBL-producing isolates exhibited about 99% sequence similarity with $bla_{CTX-M-15}$ gene that encodes β-lactamase under CTX-M group 1. One of ten amplicons exhibited 99% sequence similarity with $bla_{CTX-M-3}$, another member of CTX-M group 1. Two of the ESBL-producing isolates contain bla_{CTX-M} gene with 99% sequence similarity with $bla_{CTX-M-14}$ and $bla_{CTX-M-27}$, both under CTX-M group 9. Among the non-ESBLs, one isolate was identified to carry $bla_{CTX-M\ group\ 1}$.

Distribution of *bla* Genes Among ESBL and Non-ESBL-Producers
Both ESBL and non-ESBL-producing *E. coli* isolates exhibited highest number of isolates (13 and 27, respectively) with TEM type *bla* gene (Table 1). SHV gene was only detected from non-ESBL producing isolates and together with TEM. The CTX-M Group 1 was observed in both ESBL and non-ESBL producers but more isolates in ESBLs. CTX-M Group 1 gene was also detected together with TEM in five ESBL-producing isolates. Type CTX-M Group 9 was only detected from ESBL-producing isolates, one of which was detected together with TEM.

DISCUSSION

Antibiotic resistant ESBL and non-ESBL-producing *E. coli* are among the causative agents of infections that are often difficult to treat. Because ESBL-producing bacteria, in general, exhibit wider spectrum of antibiotic resistance and since increasing frequency of ESBLs is observed worldwide, infections with ESBL-producing strains experience treatment failures and are more difficult to control. As a result, the detection of ESBL-producing strains is deemed important. The widely used protocol to

detect ESBL-producing clinical *E. coli* isolates makes use of phenotypic evaluation using either the combination-disk diffusion assay (CLSI 2013) or the Double-disk Synergy Test (DDST, Jarlier et al. 1988). PCR detection is suggested in some reports (Dallenne et al. 2010; Ahmed et al. 2013), but more expensive and will require nucleotide sequencing to confirm results. In this study, phenotypically confirmed ESBL and non-ESBL-producing *E. coli* clinical isolates (based on resistance to β-lactam antibiotic, coupled with DDST) were subjected to plasmid DNA detection, *bla* gene detection and subsequent *bla* gene sequence analysis. These procedures were performed to detect the presence of four types of plasmid-borne β-lactamase (*bla*) gene present in each isolate. Moreover, the prevalence of four types of plasmid-borne *bla* genes among β-lactam-resistant *E. coli,* including the derivatives called ESBL, was evaluated. One main goal of the study is to determine if the routine protocol for detecting ESBL producing isolates could miss out on accurate detection of possible ESBL or presence of genes that may be potential sources of ESBL genes that may be acquired given the necessary conditions or mutations. The findings in this study highlight the significance of detecting the specific ESBL produced by the pathogen in order to monitor the emergence of ESBL derivatives, to gain information on the prevalence of specific types of *bla* genes in clinical isolates in the Philippines, and gain knowledge that could be useful for studies on spread of antibiotic resistance in different species. Data from the study are expected to contribute to information useful in developing more appropriate and effective strategies in laboratory detection of ESBL-producing strains.

Earlier studies reported that the β-lactamase gene is encoded either in the chromosomal DNA or plasmids (Schmitt et al. 2007). The prevalent ESBL-associated genes in *E. coli,* however, are reported to be plasmid-borne (Dhillon and Clark 2012).   This study was therefore focused on detecting isolates with possible plasmid-encoded ESBLs from 72 *E. coli* clinical isolates. Only one isolate did not contain a plasmid (Figure 2.A1, lane 11) and it could be hypothesized that the *bla* gene that confers antibiotic resistance in this plasmid-minus isolate is in the chromosomal DNA. A plasmid of about 23 kb was consistently observed from the profiles of all 71 isolates. The 23 kb plasmids could be containing the β-lactamase genes that confer antibiotic resistance to the ESBL and non-ESBL-producing isolates. Gram negative bacteria are described to have 6 to 160 kb plasmids carrying antibiotic resistance genes (Ogbolu et al. 2013). In general, plasmid-mediated ESBLs are observed to be encoded on large conjugative plasmids and these genes have the propensity to jump between organisms that can cause outbreaks of infections, especially if these are carried by transmissible pathogens (Dhillon and Clark 2012). In this study, the 23 kb plasmids were

detected from phenotypically β-lactamase producing *E. coli*, suggesting plasmid-mediated β-lactamase in these ESBL-producing *E. coli.* Conjugative dissemination of β-lactamase-carrying plasmids could be involved in facilitating the spread of antibiotic resistance among different members of the species.

To identify the type of β-lactamase genes in ESBL and non-ESBL-producing *E. coli* isolates, primers targeting internal fragments of plasmid-borne β-lactamase genes bla_{TEM}, bla_{SHV}, $bla_{CTX-M\ group1}$ and $bla_{CTX-M\ group9}$ (Dallenne et al. 2010) were used to amplify the target genes using plasmid DNA from each isolate as template. Results showed that bla_{TEM} (800 bp amplicon) was most prevalent among the β-lactamase genes detected for both ESBL (13/26 or 50%) and non-ESBL-producing (27/45 or 60%) *E. coli* isolates (Table 1). TEM-1 β-lactamase enzyme was the first identified plasmid-mediated β-lactamase in Gram-negative (Medeiros 1984). No bla_{SHV} gene (713 bp amplicon) was detected from any of the 26 phenotypically confirmed ESBL-producing *E. coli* isolates. The SHV β-lactamase enzyme is reported to be more often encoded in the chromosomal DNA (Bradford 2001) consistent with the observation that this gene is absent in most of the isolates studied. Another study may be focused on confirming the presence of the bla_{SHV} gene in the chromosomal DNA. It was also observed however that the bla_{SHV} gene was observed in 8 out of 45 non-ESBL-producing isolates (five showed 96 to 99% sequence similarity with same genes previously reported in ESBL-producers). Interpretation of the presence of the bla_{SHV} gene in non-ESBL-producing   *E. coli* compared to ESBLs awaits further analysis using more clinical isolates and additional sequence analysis of the genes in different isolates. Similar studies on isolates from other hospitals and other regions of the country could shed light on prevalence of bla_{SHV} gene among non-ESBL-producing *E. coli.*

The CTX-M group is considered a relatively new family of plasmid-mediated β-lactamase that preferentially hydrolyze cefotaxime (Lartigue et al. 2006). Results of this study, however, revealed that 10 of 26 (38%) ESBL-producing *E. coli* isolates have genes encoding for this enzyme, where 5 of ten were present together with bla_{TEM}. A total of 12 out of 26 ESBL-producing isolates carry bla_{CTX-M} belonging to group 1 (688 bp amplicon) and group 9 (561 bp amplicon), comparable to the prevalence of bla_{TEM} (13 of 26), suggesting a quick spread of this relatively newly detected gene among clinical isolates of *E. coli.* In the early part of 2000, CTX-M ESBL is believed to be the dominant type in East Asia (Komatsu et al. 2001; Bonnet 2004). The predominance of CTX-M type ESBL among clinical isolates has also been reported in the studies conducted in other hospitals in the Philippines (Tian et al. 2010; Lucena et al. 2012). Only one non-ESBL-producing isolate contains the CTX-M group 1

gene. This could be interpreted as a possible example of a strain that possesses the gene for ESBL that is, nevertheless inactive or unexpressed. Extensive sequence analysis of the complete gene from this isolate is necessary to confirm if the bacterium contains unexpressed ESBL gene, possibly with a mutation in the structural gene or its regulatory region. Sequence analysis of the complete gene could reveal the reason why the presence of the gene did not result to an extended-spectrum antibiotic resistance. The existence of inactive or unexpressed ESBL genes was reported from isolates of *K. pneumoniae* in China by Xu et al. (2014), indicating that "silencing" of antibiotic resistance in clinical isolates can possibly occur.

In this study, PCR using plasmids from seven ESBL-producers and 17 non-ESBL-producers (Table 1) did not result to the amplification of any of four types of β-lactamase genes. The PCR detection of β-lactamase genes in this study is limited to four pairs of primers detecting only three groups (TEM, SHV, and CTX-M) of *bla* genes. The β-lactam resistance genes in these isolates maybe in the chromosomal DNA, or could belong to other groups of β-lactamase like AmpC, PC-1, OXA, or CepA (Bush and Jacoby 2010) which would require different sets of primers for amplification. Inability to detect β-lactamase genes, if present, could also be due to sequence variation within the primer annealing sites. TEM group for example, has more than 200 bla_{TEM} genotypes, SHV group has more than 190 bla_{SHV} genotypes and more than 130 CTX-M variants have been described, on the basis of the amino acid sequences (http://www.lahey.org/ Studies). Nucleotide sequencing is therefore essential to analyze the entire gene sequence in order to accurately identify an ESBL derivative since the few point mutations could be the difference between an ESBL from a non-ESBL gene. Thus, the presence of ESBL-associated β-lactamase genes in a non-ESBL-producing *E. coli,* may serve as potential reservoirs for antibiotic resistance, as they can continuously acquire new mutations either through evolution or selective pressure as a consequence of prolonged antibiotic exposure. This study suggests that the presence of TEM and CTX-M genes even the non-ESBL-producing *E. coli* can also pose a threat in the spread of antibiotic resistance because these strains serve as reservoir of ESBL-genes that will be developed through some mutations and needs to be included in strategies for detection and efforts at prevention of spread of ESBLs.

CONCLUSION

The presence of plasmid-mediated antibiotic resistance encoded by β-lactamase genes shows that these genes can be disseminated to bacteria of the same or different species, and can play a significant role in the mobility of resistance genes. Molecular detection which revealed a high percentage of CTX-M enzymes in ESBL-producing isolates also suggests that the spread of bla_{CTX-M} gene is significantly contributing to the increase and prevalence of ESBL-producing *E. coli,* thereby complicating treatment of bacterial infections. PCR-detection of the prevalent β-lactamase gene variants could be more valuable when coupled with nucleotide sequencing to detect specific ESBL derivative, not just for monitoring but more importantly for better therapeutic strategies. More isolates need to be evaluated to confirm the molecular characteristic of the bla_{SHV} gene found in non-ESBL isolates. Moreover, the presence of ESBL-associated β-lactamase genes even in non-ESBL-producing *E. coli* suggests that these strains may serve as reservoirs for antibiotic resistance genes and may contribute to increased spread of antibiotic resistance among isolates, thereby posing a threat in the field of antibiotic therapy.

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NO CONFLICT OF INTEREST STATEMENT

There is no conflict of interest among authors, institutions, and individuals mentioned above in the conduct of this study and the preparation and submission of this manuscript.

CONTRIBUTION OF AUTHORS

Dr. Merlyn C. Cruz conceptualized the study, conducted the experiment, analyzed the data and prepared the manuscript. Dr. Cynthia T. Hedreyda contributed to data analysis and preparation of the manuscript.

REFERENCES

AHMED OI, EL-HADY SA, AHMED TM, AHMED IZ. 2013. Detection of *bla* SHV and *bla* CTX-M genes in ESBL producing *Klebsiella pneumoniae* isolated from Egyptian patients with suspected nosocomial infections. Egyptian Journal of Medical Human Genetics 14(3): 277-283.

BAUMAN RW. 2004. Controlling microbial growth in the body: Antimicrobial drugs. In: Microbiology: With diseases by body systems. RW Bauman ed. CA: Pearson Benjamin Cummings. 301-310p.

BOMASANG ES, MENDOZA MT. 2003. Prevalence and risk factors associated with extended-spectrum beta-lactamase (ESBL) production among selected *Enterobacteriaceae* isolates at the Philippine General Hospital. Phil J Microbiol Infect Dis 32(4): 151-158.

BONNET R. 2004. Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes. Antimicrob. Agents Chemother 48:1-14.

BRADFORD PA. 2001. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clinical Microbiology Reviews 14: 933-951.

BUSH K, JACOBY GA. 2010. Minireview: Updated functional classification of β-lactamases. Antimicrobial Agents and Chemotherapy 54:969-976.

[CLSI] Clinical Laboratory Standards Institute. 2013. Performance standards for antimicrobial disk susceptibility testing; Twenty-third Informational Supplement. CLSI document M100-S23 33(1): 44-49.

CRUZ M, BACANI C, MENDOZA A, HEDREYDA C. 2014. Evaluation of extended-spectrum beta-lactamase production in *Escherichia coli* clinical isolates in three hospitals in Luzon. Philippine Science Letters 7(2): 438-444.

DALLENNE C, DA COSATA A, DECRE D, FAVIER C, ARLET G. 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in *Enterobacteriaceae*. J Antimicrob Chemother 65(3): 490-495.

DHILLON RHP, CLARK J. 2012. ESBLs: A clear and present danger?. Critical Care Research and Practice. Article ID 625170 2012.

JARLIER V, NICOLAS M, FOURNIER G, PHILIPPON A. 1988. Extended spectrum β-lactamase conferring transferable resistance to new β-lactam agents in *Enterobacteriaceae*: Hospital prevalence and suspicious patterns. Review of Infectious Disease 10: 867-878.

KOMATSU, M, IKEDA N, AIHARA M, NAKAMICHI Y, KINOSHITA S, YAMASAKI K, SHIMAKAWA K. 2001. Hospital outbreak of MEN-1-derived extended spectrum beta-lactamase-producing *Klebsiella pneumoniae*. J Infect Chemother 7: 94-101.

LARTIGUE MF, POIREL L, AUBERT D, NORDMANN P. 2006. In vitro analysis of IS*Ecp1B*-mediated mobilization of naturally occurring β-lactamase gene *bla*CTX-M of *Kluyvera ascorbata*. Antimicrobial Agents and Chemotherapy 50: 1282-1286

LOTA MM, LATORRE AE. 2014. A retrospective study on extended-spectrum beta-lactamase bacteria in the Philippines from 1999-2013. Acta Medica Philippina 48(1): 28-35.

LUCENA MA, METILLO EB, OCLARIT JM. 2012. Prevalence of CTX-M extended-spectrum beta-lactamase-producing *Enterobacteriaceae* at a private tertiary hospital in Southern Philippines. Philippine Journal of Science 141(1): 117-121.

MEDEIROS AA. 1984. Beta-Lactamases. Br Med Bull 40: 18-27.

OGBOLU DO, DAINI OA, OGUNLEDUN A, TERRY ALLI OA, WEBBER MA. 2013. Dissemination of IncF plasmids carrying beta-lactamase genes in Gram-negative bacteria from Nigerian hospitals. J Infect Dev Ctries 7(5): 382-390.

PATERSON DL, BONOMO RA. 2005. Extended-spectrum beta-lactamases: A clinical update. Clin Microbiol Rev 18: 657-686.

SCHMITT J, JACOBS E, SCHMIDTT H. 2007. Molecular characterization of extended-spectrum beta-lactamases in *Enterobacteriaceae* from patients of two hospitals in Saxony, Germany. J Med Micro 56: 241-249.

TIAN GB, GARCIA J, ADAMS-HADUCH JM, EVANGELISTA JP, DESTURA RV, WANG HN, DOI Y 2010. CTX-M as the predominant extended-spectrum Beta-lactamases Among *Enterobacteriaceae* in Manila, Philippines. J Antimicrob Chemother 65(3): 584-586.

VILLANUEVA FD, TUPASI TE, ABIAD HG, BAELLO BQ, CARDAñO RC. 2003. Extended-spectrum β-lactamase production among *Escherichia coli* and *Klebsiella* spp. at the Makati Medical Center: tentative solutions. Phil J Microbiol Infect Dis 32(3): 103-108.

XU L, ZHAI Y, LYU Y, WANG Q, AN S, CHEN J, CHEN Y, LIU L, LI J, GAO Z. 2014. Identification of *Klebsiella pneumoniae* strains harboring inactive extended-spectrum beta-lactamase antibiotic-resistance genes. Chinese Medical Journal 127(15) 3051-3057.

YAZDI M, NAZEMI A, MIRINARGASI M, JAFARPOUR M, SHARIFI SH. 2012. Genotypic versus phenotypic methods to detect extended-spectrum beta-lactamases (ESBLs) in uropathogenic *Escherichia coli*. Annals of Biological Research 3(5): 2454-2458.