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Phenotypic evaluation of ESBL- and carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* from a teaching hospital in the Philippines

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Abstract. Antimicrobial resistance is a worldwide public health concern. Rise in the number of antimicrobial resistant organisms, such as extended spectrum β-lactamase- (ESBL) and carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae*, continue to burden millions of people worldwide. *E. coli* and *K. pneumoniae* were isolated and collected for four months from a teaching hospital in the Philippines. All isolates were subjected to ESBL and carbapenemase testing using the double disk synergy test and modified Hodge test, respectively. Their pattern of resistance among different classes of antimicrobial agents was also investigated using the Kirby-Bauer disk diffusion test. Among the 32 clinical isolates tested, 28.1% were positive for ESBL production and 6.3% were positive for carbapenemase production. Species-specific classification showed that *E. coli* (44.4%) has the highest rate of ESBL production whereas both *E. coli* (5.6%) and *K. pneumoniae* (7.1%) showed almost similar rates of carbapenemase production. Antimicrobial resistance pattern of drug resistant isolates showed that all organisms were resistant to ampicillin, and majority showed resistance towards ciprofloxacin, cefotaxime, ceftriaxone, and sulfamethoxazole/trimethoprim. ESBL production is seen highest among *E. coli* isolates while similar rates of carbapenemase production was observed to both *E. coli* and *K. pneumoniae* isolates. Overall, antimicrobial resistance continues to rise and poses a huge threat in public health worldwide. Efforts should be made in developing rapid tests for antimicrobial resistance and to search for effective treatment from infections caused by multidrug resistant organisms.

INTRODUCTION

Infections caused by antimicrobial resistant organisms has become a global public health catastrophe nowadays (Moxon & Paulus, 2016). Patients usually afflicted are those who are admitted in hospitals, especially those under intensive care (Lob *et al.*, 2015; Sampaio & Gales, 2016). Gram-negative bacilli, particularly those under the Family *Enterobacteriaceae*, are the most frequently isolated clinical pathogens that exhibit resistance to antimicrobial agents (Lob *et al.*, 2015; Xie *et al.*, 2017). Among all members of the Family *Enterobacteriaceae*, organisms that usually exhibit resistance include: *Escherichia coli*, *Klebsiella* spp., and *Enterobacter* spp. (Doyle *et al.*, 2012; Legese *et al.*, 2017; Lob *et al.*, 2015; Metri Basavaraj *et al.*, 2011; Xie *et al.*, 2017). Treatment of antimicrobial resistant organism has been a burden especially for both paediatric and adult patients due to the limited options of antibiotics available (Cruz *et al.*, 2014).

The main mechanism of antimicrobial resistance involves the production of hydrolytic enzymes such as β-lactamases and carbapenemases. Plasmids usually encode the genetic information necessary for this trait. These extrachromosomal DNA are the ones responsible for the easy and rapid spread of drug resistance (Cruz *et al.*, 2014;

Lucena *et al*., 2012; Moxon *et al*., 2016). One of the most important group of beta-lactamases are the extended-spectrum β-lactamases or ESBL. This enzyme is capable of degrading penicillin, first-, second-, third-generation cephalosporins, and monobactams (Cruz *et al*., 2014; June *et al*., 2016; Moxon *et al*., 2016). The production of hydrolytic enzymes by enteric bacilli confer resistance to the said antibiotics. The last resort for treating Gram-negative bacilli confirmed of ESBL production is with carbapenems. But there are also cases of enteric bacilli that can hydrolyse the so-called "drugs of last resort." The hydrolysis of carbapenems are conferred by a number of structurally similar class of beta-lactamases (Moxon *et al*., 2016). Organisms able to produce these hydrolytic enzymes are capable of degrading penicillin, cephalosporins, monobactams, and carbapenems. Carbapenems are said to be the drug-of-choice for treating ESBL-producing Gram-negative bacilli infections. Among the members of the Family *Enterobacteriaceae*, *K. pneumoniae* has been primarily associated with carbapenemase production. Since the trait is plasmid mediated, related organisms such as *E. coli* and *Enterobacter* spp. are also reported to have carbapenemase-producing properties (Kelly *et al*., 2017; Moxon *et al*., 2016; Van Duin *et al*., 2013). The rise of carbapenem-resistant Enterobacteriaceae or CRE, most commonly *E. coli* and *K. pneumoniae*, is one of the most urgent public health concerns. Due to the limited number of treatment, CRE associated mortality is between 29-52% of infected individuals (CDC, 2013; Kelly *et al*., 2017; Van Duin *et al*., 2013).

Failure to detect ESBL production among clinical isolates using laboratory methods often result to inappropriate treatment. Most clinical laboratories no longer investigate for ESBL production because methods used are too costly and complex. Dissemination of multidrug resistant strains is becoming more prevalent due to the inability of certain laboratories to detect their presence using routine laboratory susceptibility testing. Phenotypic identification methods should be routinely performed to determine the presence of ESBL production as recommended by the Clinical Laboratory Standards Institute (Poulou *et al*., 2014; The Clinical and Laboratory Standards Institute, 2016). Phenotypic testing of clinical isolates, notably *K. pneumoniae*, *K. oxytoca*, *E. coli*, and *Proteus mirabilis* using the double disk synergy test should become a routine procedure. On the other hand, carbapenemase production testing is not routinely performed in the clinical laboratory but may be executed for the purpose of epidemiological studies (The Clinical and Laboratory Standards Institute, 2016).

According to the World Health Organization (2014) report, carbapenemase-producing isolates are not well studied in developing countries, such as the Philippines. This is evident by the scarcity of data regarding these types of organisms published in peer-reviewed academic journals. The study conducted focused in determining the incidence and distribution of ESBL- and carbapenemase-producing *E. coli* and *K. pneumoniae* in a teaching hospital in the Philippines. The research also investigated the antimicrobial resistance pattern of the different clinical isolates. Studies on antimicrobial resistance will greatly improve clinical management of infections, reduce costly treatment, and decrease the impeding mortality rate among patients harbouring these types of pathogens.

MATERIALS AND METHODS

Collection, Transport and Classification of Clinical Isolates

Collection of clinical isolates was done for four months from August to November 2017. Phenotypic biochemical identification of the isolates was performed based on routine laboratory procedures.

Screening and Phenotypic Testing for ESBL Production

Kirby-Bauer disk diffusion technique using a Mueller-Hinton agar (MHA) plate was used for screening. Third-generation cephalosporin antibiotic disks (ceftriaxone, cefotaxime and ceftazidime) and a mono-

bactam (aztreonam) were aseptically impregnated onto the MHA plate. Plates were then incubated at 35-37 degrees Celsius for 16-18 hours. Zones of inhibition per antibiotic disk was measured and interpreted using the Clinical Laboratory Standards Institute M100 Guidelines to determine the pattern of susceptibility. Isolates that exhibited any degree of resistance to any of the four antibiotics were classified as "suspected ESBL-producer" (Cruz *et al.*, 2014; The Clinical and Laboratory Standards Institute, 2016). Double-disk synergy test or DDST was then performed to phenotypically confirm the isolates. Test inoculum was seeded on an MHA plate and antibiotic disks containing a beta-lactamase inhibitor (amoxicillin/clavulanic acid) is placed at the centre of the MHA plate while antibiotics containing oxyimino-beta-lactam (cefotaxime and ceftazidime) are placed at least 20-30 mm centre to centre from the amoxicillin/clavulanic acid disk. Isolates showing synergism, or the enhancement or extension of the zone of inhibition, between the disk containing oxyimino-beta-lactam and the disk containing a beta-lactamase inhibitor, were reported as ESBL-producers. Absence of the said extension between the zone of inhibition of the two disks indicates that the isolate is non-ESBL producer (Cruz *et al.*, 2014; Legese *et al.*, 2017; Lucena *et al.*, 2012; Sm *et al.*, 2013; The Clinical and Laboratory Standards Institute, 2016).

Screening and Phenotypic Testing for Carbapenemase Production

Ertapenem, a carbapenem, was impregnated on an MHA plate seeded with the prepared test inoculum. Plates were then incubated at 35-37 degrees Celsius for 16-18 hours. The zone of inhibition was measured after incubation and interpreted based on the Clinical Laboratory Standards Institute M100 Guidelines. Isolates that showed resistance to ertapenem were classified as "suspected carbapenemase-producer" (Aguirre-Quiñonero & Martínez-Martínez, 2017; Cruz *et al.*, 2014; Mathur *et al.*, 2014; The Clinical and Laboratory Standards Institute, 2016). All suspected carbapenemase-producers were tested to produce the hydrolytic enzyme using the modified Hodge test (MHT) or the cloverleaf test. A 0.5 McFarland standard suspension of indicator organism (*E. coli* ATCC 25922) was prepared using normal saline solution. Prepared suspension was further diluted with normal saline to make a dilution of 1:10 prior to seeding in an MHA plate. After the organism was streaked, ertapenem was placed at the centre of the plate. Using a sterile inoculating loop, three to four colonies of the test organism was streaked onto the plate from the edge of the antibiotic disk going to the peripheral edge of the plate. MHA plates were then incubated at 35-37 degrees Celsius for 16-20 hours. To determine carbapenemase production, MHA plates were examined for any enhanced growth around the streaked test organism. If enhanced growth was observed, isolates were reported as positive for carbapenemase production. If no enhanced growth was seen, isolates were reported as negative for carbapenemase production (Aguirre-Quiñonero *et al.*, 2017; Legese *et al.*, 2017; Mathur *et al.*, 2014; The Clinical and Laboratory Standards Institute, 2016).

Susceptibility of Isolates to Different Classes of Antibiotics

Susceptibility pattern of all clinical isolates and isolates that tested positive for ESBL and carbapenemase production were also checked. Six classes of antibiotics were used in this study, namely: penicillin (ampicillin), beta-lactamase inhibitor (amoxicillin/clavulanic acid) cephalosporins (ceftazidime, ceftriaxone, cefotaxime, cefepime), Monobactam (aztreonam), carbapenem (ertapenem), aminoglycosides (amikacin and gentamicin), Fluoroquinolones (ciprofloxacin), and a folate-pathway inhibitor (sulfamethoxazole/trimethoprim) (The Clinical and Laboratory Standards Institute, 2016; Xie *et al.*, 2017).

RESULTS

Distribution of Clinical Isolates

A total of 32 clinical isolates were isolated from both in- and out-patients of a teaching hospital in the Philippines and was used in

the study. Samples provided were randomly collected from different clinical specimens, namely: urine (15/32), blood (5/32), sputum (2/32), and wound (10/32). From the total clinical isolates, 18 (56%) were *E. coli* and 14 (44%) were *K. pneumoniae*.

Screening for ESBL and Carbapenemase Production

Approximately 34.3% of the total clinical isolates showed resistance to at least one of the third-generation cephalosporin (cefotaxime, ceftazidime, ceftriaxone) or a monobactam (aztreonam) and thus, were classified as "suspected ESBL-producers" (Table 1). Whereas, 25.0% of the total clinical isolates showed resistance to a carbapenem (ertapenem) and thus, were classified as "suspected carbapenemase-producers" (Table 2).

Phenotypic Confirmatory for ESBL and Carbapenemase Production

The double disk synergy test showed that out of the total number of suspected ESBL-producing isolates, 81.8% were positive upon phenotypic confirmatory testing (Table 1). Phenotypic confirmatory testing was also performed on the suspected carbapenemase-producing isolates and showed that only 25.0% of the suspected clinical isolates were positive for carbapenemase production via the modified Hodge test (Table 2). Isolates that tested positive for carbapenemase production also tested positive for ESBL production.

Species-specific classification were also noted and summarized in Tables 2 and 3. From the confirmed ESBL isolates, 44.4% were *E. coli* and 7.1% were *K. pneumoniae*. Whereas, 5.6% of *E. coli* and 7.1% of *K.*

Table 1. Screening and Phenotypic Confirmatory Test Results for ESBL Production

Organism	Suspected ESBL-producer	Phenotypically Confirmed ESBL-producer	Incidence
E. coli	10 out of 18 (55.6%)	8 out of 10 (80.0%)	44.4%
K. pneumoniae	1 out of 14 (7.1%)	1 out of 1 (100.0%)	7.1%
Total Isolates	11 out of 32 (34.3%)	9 out of 11 (81.8%)	28.1%

Table 2. Screening and Phenotypic Confirmatory Test Results for Carbapenemase Production

Organism	Suspected Carbapenemase-producer	Phenotypically Confirmed Carbapenemase-producer	Incidence
E. coli	6 out of 18 (33.3%)	1 out of 6 (16.7%)	5.6%
K. pneumoniae	2 out of 14 (14.3%)	1 out of 2 (50.0%)	7.1%
Total Isolates	8 out of 32 (25.0%)	2 out of 8 (25.0%)	6.3%

Table 3. Antimicrobial Resistance Pattern of Clinical Isolates

	Antimicrobial Resistance Pattern (%)											
Clinical Isolates	AM	AK	CN	CIP	CAZ	CTX	CRO	FEP	SXT	AMC	ATM	ETP
E. coli	100	16.7	44.4	66.7	33.3	44.4	44.4	33.3	83.3	50.0	22.2	33.3
K. pneumoniae	100	7.1	21.4	7.1	7.1	7.1	7.1	7.1	50.0	21.4	7.1	14.3
Total Isolates	100	12.5	34.4	40.6	21.9	28.1	28.1	21.9	68.8	37.5	15.6	25.0

AM: Ampicillin, AK: Amikacin, CN: Gentamicin, CIP: Ciprofloxacin, CAZ: Ceftazidime, CTX: Cefotaxime, CRO: Ceftriaxone, FEP: Cefepime, SXT: Sulfamethoxazole/Trimethoprim, AMC: Amoxicillin/Clavulanic acid, ATM: Aztreonam, ETP: Ertapenem.

pneumoniae isolates were observed to produce carbapenemase using the modified Hodge test.

Antimicrobial Resistance Pattern of Clinical Isolates

Table 3 shows the overall resistance pattern of clinical isolates. High resistance rate was seen towards ampicillin (100%), sulfamethoxazole/trimethoprim (68.8%), and ciprofloxacin (40.6%). On the other hand, amikacin and aztreonam showed an overall resistance rate of 12.5% and 15.6%, respectively. Whereas, ceftazidime and cefepime both showed a resistance rate of 21.9%.

Species specific resistance were also noted and emphasized in the study. Majority of *E. coli* isolates showed high rates of resistance towards ampicillin (100%) and sulfamethoxazole/trimethoprim (83.3%). Low resistance rates were observed towards both amikacin (16.7%) and aztreonam (22.2%). All isolates of *K. pneumoaniae* exhibited resistance to ampicillin and 50% showed resistance towards sulfamethoxazole/trimethoprim. Relatively low resistance rates were observed towards amikacin, ciprofloxacin, ceftazidime, cefotaxime, ceftriaxone, cefepime, and aztreonam.

Antimicrobial Resistance Pattern of ESBL- and Cabapenemase-producing Isolates

The overall resistance pattern of both ESBL- and carbapenemase-producing isolates are summarized in Figure 1. All ESBL- and carbapenemase-producing isolates showed resistance to ampicillin while 90.9% displayed resistance to ciprofloxacin, cefotaxime, ceftriaxone, and sulfamethoxazole/trimethoprim. However, only 27.3% of both ESBL- and carbapenemase producer showed resistance to amikacin.

Pattern based on the mechanism of drug resistance was also compared and summarized in Figure 2. Carbapenemase-producers showed higher rates of antimicrobial resistance compared to ESBL-producers. Overall, carbapenemase-producing isolates were resistant to 11 out of the 12 antibiotics used in the study. Whereas, all the ESBL-producers showed resistance towards ampicillin, and majority were resistant to ciprofloxacin, cefotaxime,

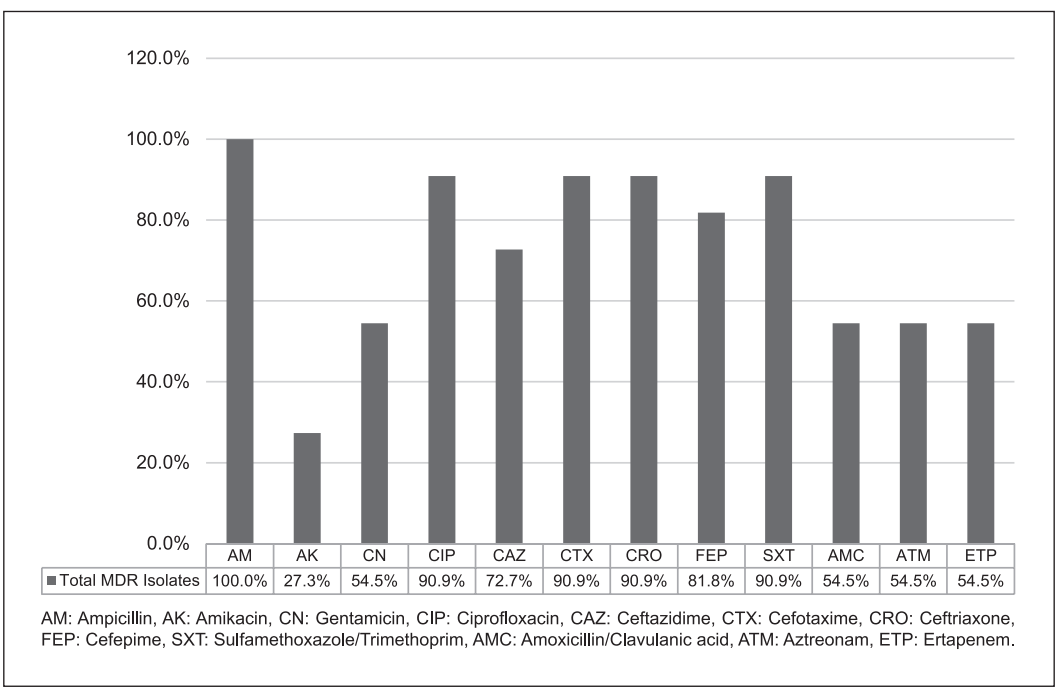
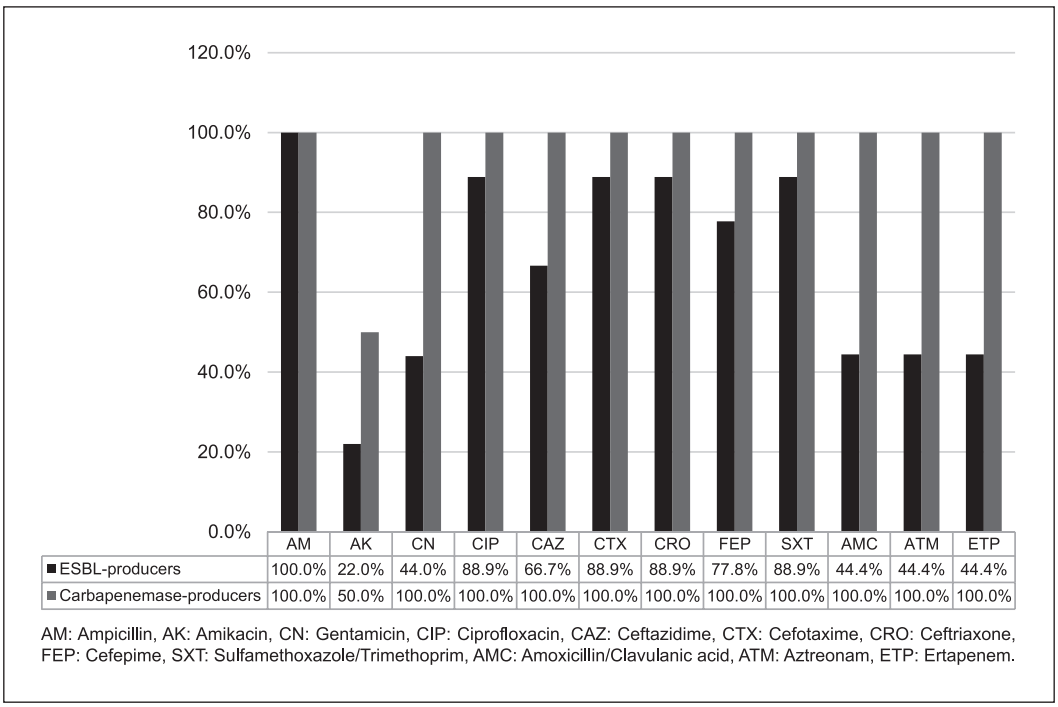


Figure 1. Antimicrobial resistance pattern of multidrug resistant isolates.



	AM	AK	CN	CIP	CAZ	CTX	CRO	FEP	SXT	AMC	ATM	ETP
ESBL-producers	100.0%	22.0%	44.0%	88.9%	66.7%	88.9%	88.9%	77.8%	88.9%	44.4%	44.4%	44.4%
Carbapenemase-producers	100.0%	50.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

AM: Ampicillin, AK: Amikacin, CN: Gentamicin, CIP: Ciprofloxacin, CAZ: Ceftazidime, CTX: Cefotaxime, CRO: Ceftriaxone, FEP: Cefepime, SXT: Sulfamethoxazole/Trimethoprim, AMC: Amoxicillin/Clavulanic acid, ATM: Aztreonam, ETP: Ertapenem.

Figure 2. Comparison of the antimicrobial resistance pattern of ESBL- and carbapenemase-producing isolates.

ceftriaxone, and sulfamethoxazole/trimethoprim.

DISCUSSION

Phenotypic vs. Genotypic Bacterial Characterization

The present study relied on the phenotypic bacterial characterization based on ESBL and carbapenemase production. Although genotypic identification has already emerged as an alternative or complementary method to phenotypic approach, many laboratories in developing countries like the Philippines, still rely on conventional phenotypic identification. The lack of infrastructure for infectious disease management, limited resources and skilled staff are just some of the problems encountered by laboratories in low resource settings. In fact, some institutions even lack microbiology facilities (Ahmed *et al*., 2015; Ulu-Kilic *et al*., 2013).

Phenotypic testing is considered as the gold standard in formulating microbial treatment decision. However, genotyping may replace such method because of its greater specificity and sensitivity especially in detecting ESBL-producing strains (Krishnamurthy *et al*., 2013). But to totally replace phenotypic bacterial characterization with genotypic approach alone is not a recommended method. The natural versatility of phenotypic testing as well as its cost-effectivity and ease still make it important in therapeutic decision making and a huge part of the microbiology laboratory practice. In the study of Gautier *et al*. (2018), the researchers stated that phenotypic evaluation methods could be used as an alternative to molecular testing while Bart & Lee (2018) suggested to use genotyping to supplement the result of the traditional approach especially with Gram-negative organism where the absence of resistant gene may not necessarily indicate the susceptibility to a particular drug (Bard & Lee, 2018). In addition, molecular methods alone are not capable of establishing distinct boundaries among phylogenetically related species. Thus, both genotypic and phenotypic identification are essential in laboratory

testing (Donelli *et al*., 2013). In the current study, the lack of a molecular diagnostic laboratory in the locale contributed to its limitation. Thus, it is suggested that future researches within the same locale use both approaches for improved bacterial characterization.

ESBL- and Carbapenemase-producing *E. coli* and *K. pneumoniae*

With the increasing resistance of micro-organisms specifically Gram-negative *Enterobacteriaceae* like *E. coli* and *Klebsiella* spp., the emergence of ESBL and CRE has become a public health concern. Data reported to the Center for Disease Control and Prevention (CDC) from 2007 indicates an 8% alarming increase of *K. pneumoniae* carbapenem resistance compared with only <1% in 2000 (Hirsch & Tam, 2010). Incidence of antimicrobial resistance usually vary from country-to-country and between regions. For children, those who are in greater risk include patients under immunosuppressive treatment and with invasive medical devices like catheters. A surveillance program known as Study of Monitoring Antimicrobial Resistance Trend gathered data from five countries (India, Israel, Spain, USA and Greece) on children with CRE infections. The survey showed that India has the highest number of antimicrobial resistance cases and three major isolates were found, namely, *Enterobacter* spp., *K. pneumoniae* and *E. coli*.

Exposure to antimicrobials and health care are among the most important risk factors for CRE infections. Isolates from intensive care units showed the highest rate of antimicrobial resistance compared to other wards in the hospital (Xie *et al*., 2017). In a study by Gupta *et al*. (2011), results show that carbapenem resistant *K. pneumoniae* are associated with organ transplantations, use of mechanical ventilation, use of anti-microbials and longer period of stay in the hospital especially in the intensive care. Similar findings were noted with the study of Lob *et al*. (2015) where *K. pneumoniae* isolates from intensive care patients exhibited the highest percentage of antimicrobial resistance phenotypically. *Klebsiella* spp. has the ability to spread quickly in hospital environment and contribute to its resistance along with the mismanagement of antibiotic in hospitals where most of the multidrug resistant organisms are isolated (van Duin & Paterson, 2016).

In the study, the overall incidence of ESBL and carbapenemase production among the tested clinical isolates were 28.1% and 6.3% respectively. Samples came from urine, blood, sputum and wound specimens submitted by both in and out-patients of the teaching hospital. Out of the isolates tested, *E. coli* showed the highest rate of ESBL production whereas, almost similar rates of carbapenemase production was observed with both *E. coli* and *K. pneumoniae*. This observation is in contrast with the study of Sampaio *et al*. (2016), Boix-Palop *et al*. (2016), and Abdulhaq & Basode (2015) where *K. pnuemoniae* was the documented predominant species that exhibited both ESBL and carbapenemase production phenotypically in Brazil, Saudi Arabia and Spain, respectively. In Karachi, the highest frequency of phenotypically confirmed ESBL-producers was observed from organisms under the Genus *Enterobacter* (June *et al*., 2016).

Studies conducted in the Philippines presented similar results. *E. coli* has the highest rate of ESBL production compared to *K. pneumoniae*. Cruz *et al*. (2014) reported that approximately 23% of the *E. coli* isolates in three hospitals in Luzon, Philippines showed ESBL production phenotypically while another study in Southern Philippines showed approximately 5.1% of the enteric Gram-negative bacilli isolates showed ESBL production by both phenotypic and genotypic means (Lucena *et al*., 2012). These studies also showed that the main organism under Family *Enterobacteriaceae* that exhibits ESBL production is *E. coli*, followed by *K. pnuemoniae* and *Enterobacter* spp. (Cruz *et al*., 2014; Lucena *et al*., 2012).

Phenotyping of the bacterial isolates revealed that the rate of carbapenemase production was 6.3%, which is lower compared to the study of Begum & Shamsuzzaman (2016) (80%) but higher

compared to the study of Eshetie *et al.* (2015) (2.73%), both of which used phenotypic techniques. Factors such as geographical distribution of isolates, population, target size, and the methodology used contribute to the variation of prevalence among the different studies. This observation is supported by both Kristiansson *et al.* (2009) and Okeke *et al.* (1999), where results show that perceived socioeconomic and behavioural factors such as poverty, self-medication, and treatment non-compliance contribute to antibiotic resistance. Individuals coming from low- and middle-income countries are the ones prone to such behaviour. Other reasons for antimicrobial resistance seen among healthcare facilities and professionals in low- and middle-income countries are due to overcrowding of patients, inadequate prescription, overprescribing and improper selection of antibiotics (Basu *et al.*, 2008; Patel *et al.*, 2005). Kumar *et al.* (2008) also stated that improper diagnosis and inadequate prescription due to lack of microbiology facilities and the irrational prescription by unauthorized practitioners are also responsible for resistance development.

Antimicrobial Resistance Pattern and Treatment Option

In the present study, all clinical isolates were resistant to ampicillin and majority showed high degree of resistance towards sulfamethoxazole/trimethoprim (68.85%). Whereas, all ESBL- and carbapenemase-producing isolates showed resistance to ampicillin and majority (90.9%) were resistant to the third generation cephalosporins (cefotaxime and ceftriaxone), ciprofloxacin, and sulfamethoxazole/trimethoprim. These results are similar with the study conducted in Guangzhou, China where researchers have shown that members of Family *Enterobacteriaceae* are resistant towards third-generation cephalosporins. In their study, 11 out of 23 or approximately 47.8% of the isolates showed resistance to third-generation cephalosporins (June *et al.*, 2016). Other researches, most notably Mutiibwa and Tumusiime (2013), Chaudhuri *et al.* (2011) and Seni *et al.* (2016), showed that majority of ESBL isolates have a high degree of resistance (>80%) to commonly used antibiotics.

The present study also revealed the potential antibiotics that can be used as treatment for ESBL- and carbapenemase-producing isolates. Based on the results, only 27.3% of the clinical isolates were resistant to the aminoglycoside – amikacin. Similar findings were noted by Hirsch and Tam (2010), with high treatment success rates for aminoglycosides (75%), polymyxin combinations (73%), and tigecycline (71%). In contrast, carpenems (40%) and polymyxin monotherapy (14%) should no longer be used due to their low success rates in managing resistant strains. Many studies also agree with the results of the researchers indicating the potential use of amikacin alone or in combination with other antibiotics as a treatment option for ESBL- and carbapenemase-producing clinical isolates (Endimiani *et al.*, 2009; Maltezou *et al.*, 2009; Nadkarni *et al.*, 2009; Weisenberg *et al.*, 2009).

CONCLUSION

The study was able to determine that the incidence of ESBL and carbapenemase-producing *E. coli* and *K. pneumoniae* continues to rise and pose a public health concern. Antimicrobial resistance pattern of the different clinical isolates confirms multidrug resistance with "very high" resistance patterns observed in common antibiotics, such as, ampicillin, ciprofloxacin, cefotaxime, ceftriaxone, and sulfamethoxazole/trimethoprim. Despite the relatively less sensitive and less specific nature of phenotypic assays in comparison to genotypic assays, it is still evident that empirical prescription of the drugs should be lessened. Studies regarding treatment for infections caused by these organisms should be given priority due to the limited antibiotics available in the global market. Furthermore, to make treatment regimens more effective, studies regarding the development of more

rapid and accurate tools in detecting antimicrobial resistance should also be conducted.

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