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Extended Spectrum β-Lactamases among *Enterobacteriaceae* Isolated from Clinical Specimens of a Tertiary Hospital in 2014

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Authors' contributions

This work was carried out in collaboration among all authors. Author RRT designed the study and wrote the protocol with author ILPN who also performed literature research and wrote parts of the manuscript with author AMNG. The collection of data, statistical analysis and the first manuscript draft was done by authors KBR, RRT and MDMG. All authors read and approved the final manuscript.

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ABSTRACT

Extended spectrum beta-lactamase producing *Enterobacteriaceae* have become widespread among hospitals and even in the community. Such phenomenon poses a therapeutic dilemma against certain antimicrobial drugs that limit therapeutic options. This study was conducted to evaluate the prevalence of ESBL producing *Enterobacteriaceae* in a tertiary hospital in Bacolod City and their antibiotic resistance profile for the year 2014. Out of 662 isolates, 125 (18.89%) were found to be ESBL producing and were frequently seen in *Escherichia coli* (4.96%), *Klebsiella pneumoniae* (4.08%) and *Enterobacter aerogenes* (3.36%). Urine was found to be the most common specimen to contain this type of bacteria (7.70%), followed by wounds and abscess (5.14%), and sputum (3.63%). These isolates were also found to be least resistant to amikacin with 10.5% followed by

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imipenem and meropenem with 17.1% and 15.4% respectively. Furthermore, there was no significant difference with regard to sex, but with significant difference in terms of age.

Keywords: Extended spectrum β-lactamases; Enterobacteriaceae; tertiary hospital; Bacolod city.

1. INTRODUCTION

Resistance of pathogenic organisms to countenance antibioticshas becomes a worldwide problem with serious consequences on the treatment of infectious diseases [1]. The heightened use or misuse of antibiotics in human medicine is primarily contributing to the phenomenon. Because of this, there is an alarming increase of antibiotic resistance in bacteria that cause either community infections or hospital acquired infections. Antimicrobial-resistant infections currently claim approximately 700,000 lives each year with at least 50,000 of it across Europe and US alone, and hundreds of thousands more dying in other areas of the world.Furthermore, studies estimate that 300 million people worldwide are expected to die prematurely because of drug resistance over the next 35 years [2].

One of the major factors in drug resistance is the production of enzymes like the ESBL. According to Rawat et al. [3], ESBL are group of plasmid-mediated, diverse, complex and rapidly evolving enzymes that are posing a major therapeutic challenge today in the treatment of hospitalized and community-based patients. ESBLs are a rapidly evolving group of β-lactamases which share the ability to hydrolyze third-generation cephalosporins and aztreonam but are inhibited by clavulanicacid.Most infections caused by these organisms are described as nosocomial or healthcare related, but has been observed to spread in the community [4]. For the reason that local data is not available, this study was initiated to give baseline information and to describe the presence of ESBL producing *Enterobacteriaceae* in the area.

2. MATERIALS AND METHODS

2.1 Inclusion/Exclusion Criteria

Data on culture and antibiotic susceptibility tests were collected from the tertiary hospital from January 2014 to December 2014, after the grant of permission from the laboratory supervisor. Data such as age, sex, specimen source, ID of the isolated *Enterobacteriaceae* and susceptibility testing were included. No personal data was retrieved from patients in order to preserve confidentiality.

2.2 Detection of Extended Spectrum Beta-Lactamase

Screening and confirmatory tests for production of Extended Spectrum Beta-Lactamase (ESBL) were determined using the antibiotic discs Ceftazidime (30 μg), Aztreonam (30 μg), Cefotaxime (30 μg), Ceftriaxone (30 μg), Cefotaxime-clavulanic acid (30/10 μg), and Ceftazidime-clavulanic acid (30/10 μg) as mentioned in the M100-S23 [5]. Screening of ESBL was made by observing thezones of inhibition that are equal to or lower than any isolate with a zone of 22 mm for ceftazidime, 27 mm for aztreonam and cefotaxime, and 25 mm for ceftriaxone. Isolates that fell in the aforementioned screening criteria were then phenotypically confirmed using disc potentiation assay utilizing ceftazidime, cefotaxime, cefotaxime-clavulanate discs, and ceftazidime-clavulanate discs. A 5 mm increase in zone diameter of either antimicrobial agent tested in combination with clavulanateversus the zone diameter of the agent when tested alone confers ESBL production [6].

2.3 Statistical Analysis

The gathered data were analyzed statistically to determine the frequency, resistance profile and distribution of ESBL producing *Enterobacteriaceae* among patients in terms of age and sex using WHONET version 5.6 software downloaded from World Health Organization website. Age was divided into two categories, adult (19 years old and above) and pediatric (0 – 18 years old) automatically by the WHONET software.

3. RESULTS

From January 2014 to December 2014, a total of 662 *Enterobacteriaceae* were isolated and identified to genus and species level from different clinical specimens. *E. coli* was the most frequent isolate having a total of 256 (38.67%),

followed by *K. pneumoniae* with 107 (16.16%) and *E. aerogenes* with 57 (8.61%). From these 662 isolates, 125 (18.89%) were observed to produce the enzyme ESBL and noted to be the most numbered producer is *E. coli* (24.8%), followed by *K. pneumoniae* (20.72%) and *E. aerogenes* (3.17%) as shown in Table 1.

For the distribution of bacterial isolates and ESBL producers as to the sample source, urine was found to be the major source with 42.60% of the total specimen, followed by wounds and abscess with 29.76%. Out of 125 ESBL producing isolates, it was observed that it was frequently found in urine with 40.8%followed by wounds and abscesses with 27.2%. Table 2 summarizes the specimen wise distribution of ESBL producers.

As to the distribution of isolates with regard to specimen source, *E. coli* was still the most common bacteria isolated from urine, wounds and abscesses, and blood specimens while *K. pneumoniae* was frequently found in sputum and ETA. Table 3 shows the frequency of the isolated *Enterobacteriaceae* per specimen.

The age and sex wise distribution of the ESBL producers which is shown in Table 4, revealed that the maximum number of ESBL producers were seen in the adult age group and that the prevalence was more among the females (the highest) comprising 48% of the 125 ESBL producing strains than among the males covering 42% of the total ESBL producing strains. The least prevalence was seen in the last two groups; the pediatric males and pediatric females.

Based on the gathered data, Trimethoprim/Sulfamethoxazole had the highest resistance with 78.4%, followed by ampicillin/sulbactam and ciprofloxacin with 75% and 74.4% respectively. Amikacin was most effective with 10.5% resistance rate followed by meropenem and imipenem with 15.4% and 17.1%.

Table 1. Incidence of identified *Enterobacteriaceae* and ESBLs among the recovered isolates

Organisms	Number of isolates	%	ESBL producers	% of ESBL producers
E. coli	256	39	31	12
K. pneumonia	107	16	27	25
E. aerogenes	57	9	21	37
P. mirabilis	33	5	3	9
P. agglomerans	28	4	7	25
E. gergoviae	24	4	4	17
E. cloacae	22	3	7	32
K. ozaenae	22	3	6	27
P. vulgaris	20	3	1	5
E. hermannii	18	3	4	22
C. koseri	16	2	3	19
H. alvei	16	2	4	25
Salmonella sp.	12	2	0	0
C. freundii	11	2	4	36
E. tarda	11	2	2	18
S. marcescens	4	1	0	0
K. oxytoca	3	0	0	0
P. penneri	1	0	0	0
P. rettgeri	1	0	1	100
Total	662	100	125	19

Table 2. Specimen wise distribution of ESBL producers

Type of specimen	Number of isolates	ESBL producers	(%)
Urine	282	51	18
Wound & Abscess	197	34	18
Blood	88	13	15
Sputum & ETA	95	24	25
Total	662	125	17

Table 3. Distribution of bacteria as to specimen source

Organism	Urine	Blood	Sputum & ETA	Wound & abscess	Total
C. freundii	3	3	2	3	11
C. koseri	4	2	1	9	16
E. tarda	4	2	1	4	11
E. aerogenes	15	3	19	20	57
E. cloacae	7	1	5	9	22
E. gergoviae	2	5	3	14	24
E. coli	151	45	10	50	256
E. hermannii	14	1	1	2	18
H. alvei	6	3	3	4	16
K. oxytoca	1	0	1	1	3
K. ozaenae	12	1	7	2	22
K. pneumoniae	35	9	36	27	107
P. agglomerans	15	0	2	11	28
P. mirabilis	8	0	4	21	33
P. penneri	0	0	0	1	1
P. rettgeri	1	0	0	0	1
P. vulgaris	3	0	0	17	20
Salmonella sp.	0	12	0	0	12
S. marcescens	1	1	0	2	4
Total	282	88	95	197	662

Table 4. Age and sex wise distribution of the ESBL producers

Organism	Pediatric		Adult	
	Male	Female	Male	Female
C. freundii	1	0	1	2
C. koseri	1	0	0	2
E. tarda	0	0	0	2
E. aerogenes	1	1	8	11
E. cloacae	1	0	3	3
E. gergoviae	0	0	4	0
E. coli	1	2	9	19
E. hermannii	0	0	0	4
H. alvei	0	0	2	2
K. oxytoca	0	0	0	0
K. ozaenae	0	0	3	3
K. pneumoniae	1	1	17	8
P. agglomerans	1	1	4	1
P. mirabilis	0	0	0	3
P. rettgeri	0	0	1	0
P. vulgaris	0	0	1	0
S. marcescens	0	0	0	0
Total	7	5	53	60

Table 5. Significant difference of ESBL-positive organisms as to selected variables

Variable	Category	*P*-value	Interpretation
Age	Pediatric (0-18 yrs old) adult (19 and above)	0.01	Significant
Sex	Male	0.05	Not significant
	Female		

Out of the total 662 isolates, *E. coli* was noted to be the most common of the said bacteria that produces the enzyme ESBL, followed by *K. pneumoniae* and *Enterobacter species* with frequencies of 24.8%, 21.6% and 16.8% respectively. This is because *Entero bacteriace*ae can harbor plasmid-mediated ESBL genes and both *E. coli* and *K. pneumoniae* are

the most common ESBL positive species [7]. This type of isolates that produce the said enzyme is clinically relevant and remains an important cause of cephalosporin therapy failure [8]. This finding, as supported by Ali et al. [9], is accounted to the said organisms' emerging resistance to agents such as extended-spectrum cephalosporins, mono bactams, carbapenems and ß-lactam–ß-lactamase inhibitor combinations through the production of a variety of ß-lactamases, alterations in the penicillin-binding proteins, outer membrane permeability and combinations of multiple mechanisms of resistance [10].

In the current study, *E. coli* is noted to be themost prevalent pathogen isolated in wound and abscess, urine and blood taking into consideration that *E. coli* is a member of the human microbiota that can cause several kinds of nosocomial and community acquired infections [10]. *E. coli* is known to invade urothelial cells and form quiescent intracellular bacterial reservoirs [11]. This is followed by *K. pneumoniae* that causes serious respiratory tract infections and is also one of the frequent pathogen causing urinary tract infections [4]. Both *K. pneumoniae* and *E. coli* remain as the major ESBL producing organism worldwide and are recommended to be tested routinely for ESBL production [12]. This data is also at par with the data of Lenhard-Vidal et al. [10] in Brazil, Jabeen et al. [13] in Pakistan and Ghafourian et al. [14] in Iran.

Urine was observed to be the most common specimen source that contains ESBL producing species in this study. This is because recurrence of urinary tract infection is common even for a healthy individual [11] and a common problem of women throughout their lifetimes [15]. Statistically, urinary tract infection is the most common bacterial infection that counts for more than 8 million clinic visits and 1 million emergency department visits each year [16]. Furthermore, urinary tract infection is twice as common among women of all ages compared with men. Females have the highest prevalence of ESBL compared to males because they are more prone to urinary infections, necessitating repeated use of antibiotics [15,17]. Moreover, UTI is also very common both in the community and hospitalized patients that ranks high amongst the most common reasons that compel a patient to seek medical attention [7]. Urinary tract infection can range from asymptomatic bacteriuria, to symptomatic and recurrent, and finally, sepsis [16].

As to age group, the maximum number of ESBL producers was seen in the adult age group and that the prevalence was more among the females comprising 48% of the 125 ESBL producing strains. ESBLs-producing gram-negative rods were most frequent at latter part of life where they most commonly infect individuals due to exposure to antibiotics and other factors [18]. The least prevalence was seen in the last two groups; the pediatric males and pediatric females. Pediatric males comprise 6% of the total ESBL positive isolates. Out of 125 ESBL positive isolates, only 4% of the pediatric females are found to be ESBL producers. Hence, the pediatric females have the lowest prevalence of ESBL as to this study. According to Paterson and Bonomo [5], the age distribution of β-lactamase and ESBLs producers among the patients showed high prevalence among the adult age group. This may be due to the fact that older people often become immune-compromised and stand greater chances of coming down with infections. Such infections are of many types but in patients with low immunity they are more difficult to treat. This may explain the relatively high presence of extended spectrum *beta-lactamase* producers among the aged [19].

The top 5 antibiotics where ESBL-producing *Enterobactericeae* showed highest resistance against were trimethoprim/ sulfamethoxazole (78.4), ampicillin/ sulbactam (75%) and ciprofloxacin (74.4%). It is not surprising that antibiotic choice for infections with such ESBL-producing organisms is seriously reduced given the ability of such organisms to hydrolyze many β-lactam antibiotics [5]. This resultmay have been caused by frequent antibiotic use over long periods of time which in turn, puts selective pressure on bacteria, and causes resistance to spread. Since many antibiotics are used too often or incorrectly, it is expected that resistance would spread faster than it would naturally [19]. Additionally, the three aforementioned antibiotics are considered to be the least effective to use as medications in ESBL-producing organisms because a high resistance rate is an indication that the organism is no longer susceptible to the said drug, making treatment ineffective and null.

Meanwhile, the ESBL-producing organisms were least resistant to amikacin, meropenem, and imipenem. Amikacin had 10.5% resistance rate followed by meropenem with 15.4% resistance rate and imipenem which has 17.1% resistance rate. This is an implication that the organisms in the study were most susceptible to the three

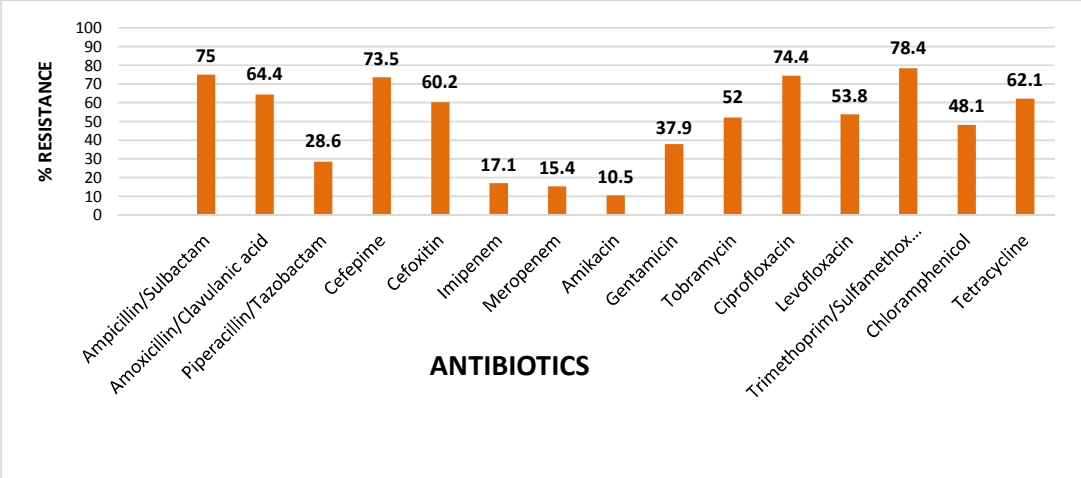

Fig. 2. Antibiotic resistance rates (%) of ESBL-producer

aforementioned antibiotics. This finding is also similar to the study of Juayang et al. [6], Maina et al. [8] and Al Muharrmi et al. [20]. Amikacin has good *in vitro* activity against ESBL-producing organisms such as *K. pneumoniae* strains [21]. In vitro, the carbapenems have the most consistent activity against ESBL-producing organisms, given their stability to hydrolysis by ESBLs. This implies that ESBLs are generally inactive against the carbapenems [5]. With this, Pitout & Laupl and [22] and Juayang et al. [6], indicated that carbapenems or amikacin are widely regarded as the drugs of choice for the treatment of severe infections caused by ESBL-producing *Enterobacteriaceae*. Unfortunately, even though amikacin was noted to be the most effective, it has toxic side effects such as nephrotoxicity and neurotoxicity, making carbapenems and piperacillin/tazobactam as the treatment of choice as of this time [6]. These results are analogous to that of Jabeen et al. [13] which showed results of higher resistance to classes of antibiotics such as aminoglycosides and quinolones and no resistance to carbapenems.

The current study implies difficulty of treating infections caused by ESBL producing bacteria taking into consideration that 80% of the antibiotics tested exceeded the 20% resistance rate. This then makes the remaining 80% of the antibiotics tested inappropriate for empiric use during the time of this study. Carbapenems are also nearing the 20% mark of resistance making these last of the line antibiotics probably less useful in the upcoming years unless good antimicrobial stewardship and interventions are formulated.

4. CONCLUSION

ESBL producing *Enterobacteriaceae* displays high levels of antimicrobial resistance that hinders efficacy of cephalosporins and other related drugs, thus, making carbapenem as the drug of choice for treatment. *E. coli* was observed to be the most frequent ESBL producer followed by *K. pneumoniae* that are commonly isolated in urine, wound and abscess, and even respiratory specimens that presents mostly in adults. It is therefore imperative that monitoring of ESBL producing organisms among hospitals to evaluate local status, stricter antimicrobial stewardship and for formulation of policies for empiric therapy.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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