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Detection of Betalactamase Producing Bacterial Genes and their Clinical Features

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Abstract: The present study was aimed to identify the gene of drug resistance betaclamase producing bacteria and clinical features of the infected patients at Hospital University Kebangsaan Malaysia. Blood samples from the patients were collected, processed and betalactamase producing drug resistance bacteria were identified by antibiotic sensitivity testing. Genes of the drug resistance bacteria were detected and characterized by polymerase chain reaction. A total of 34 isolates of drug resistance Betalactamase producing *E.coli* and *Klebsiella* spp. were isolated from 2,502 patients. Most common drug resistance gene TEM was found in 50% of the isolates. 11% was found positive for both TEM and SHV. Next 11% of the isolates expressed only SHV genes. Clinical features of the patients were recorded from where the bacteria isolated. Regarding community affiliations 70.5% of the infected patients were Malay 17.6% were Indian and 11.7% were Chinese. Majority of the patients has an underlying pre-morbid condition as reflected by their diagnosis. Better infection control and hygiene in hospitals, plus controlled and prudent use of antibiotics, is required to minimize the impact of drug resistance betalactamase producing bacteria and the spread of infections.

Key words: Extended spectrum betalactamase, drug resistance, *Escherichia coli*, *Klebsiella*, polymerase chain reaction

INTRODUCTION

Extended Spectrum Betalactamase (ESBLs) are capable of conferring bacterial resistance to penicillin, first second and third generation cephalosporins and aztreonam by hydrolysis of these antibiotics (Hashim *et al*., 2009). ESBLs are bacteria produce enzymes that mediate resistance to extended-spectrum (third generation) cephalosporins (e.g., ceftazidime, cefotaxime and ceftriaxone) and monobactams (e.g., aztreonam) but do not affect cephamycins (e.g., cefoxitin and cefotetan) or carbapenems (e.g., meropenem or imipenem). The presence of an ESBL-producing organism in a clinical infection can result in treatment failure if one of the above classes of drugs is used. ESBLs can be difficult to detect because they have different levels of activity against various cephalosporins. Thus, the choice of which antimicrobial agents to test is critical. The wide spread use of third generation antibiotics is believed to be the major cause of mutation in the enzymes that has led to the emergence of extended spectrum betalectamase producing bacteria (Nathisuwam *et al*., 2001). ESBLs is now problem in the hospitalized patients worldwide. In Malaysia, the incidence of hospital acquired infection due to ESBL producing organisms are increasing. The majority of ESBL producing strains are *K. pneumoniae*, *K. oxytoca* and *E. coli* (Hashim *et al*., 2009).

A study was conducted on the prevalence of organisms' resistance to beta-lactam and non-β-lactam antibiotics (Ash *et al*., 2002) However, in the study genes of drug resistance bacteria were not identified by molecular assay. On the other hand, different authors (Diederen and Euser, 2009; Livermore and Woodford, 2006; Murk *et al*., 2009) carried out research on the detection of betalactamase producing bacteria from patients, however, clinical features of the patients infected with the organisms were not carried out which are necessary for the clinicians to identify the nature of drug resistance bacterial infections. In Malaysia, the incidence of hospital acquired infection due to ESBL producing organisms are also increasing. The majority of ESBL producing strains are *K. pneumoniae*, *K. oxytoca* and *E. coli* (Hashim *et al*., 2009). Keeping these in view the present study was, therefore, aimed at to identify the gene of drug resistance betaclamase producing bacteria and clinical features of the patients from where the bacteria

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were isolated and identified. The study would provide guidance for the clinicians to identify the patients those might have infected with drug resistance bacteria.

MATERIALS AND METHODS

Study area: This study was conducted during 2007 to 2008 at the Department of Medical Microbiology and Immunology, Faculty of Medicine, National University Malaysia, Cheras-56000, Kula Lumpur, Malaysia. All the samples were collected from the patients of Hospital Kuala Lumpur, which has 82 wards with 2,502 beds and is the largest government tertiary referred hospital, primarily focus on public services.

Study design: This is a descriptive study, design to demonstrate ESBL producing bacteria from the patients showing the symptoms of bacteremia. A total of 34 isolates were selected from blood culture of patients who had the evidences of such symptoms out of 2502 patients examined. Blood cultures were processed using the BECTEC 9240 system (Becton Dickinson, USA). Clinico-epidemiological features of the patients from where ESBLs were recorded.

Bacteria identification and susceptibility test: Blood samples collected from the patients were processed and identified as per the methods described by Cheesbrough (2006). The microbial susceptibility tests were carried out using the disc diffusion method done on Mueller-Hinton agar plate, which is inoculated, with a suspension, adjusted to 0.5 McFarland turbidity standards. The plates were incubated overnight and susceptibility was defined according to CLSI Gidelines (2006).

Detection of drug resistance bacterial genes: Betalactamase producing bacteria were screened by antibiotic sensitivity pattern. Genes of the bacteria were identified by PCR as per the method of Gruteke *et al.* (2003) using established primers for TEM and SHV genes that are responsible for drug resistance. After amplification of the genes the PCR products were documented.

Clinical features: A total 34 from patients from where ESBL bacteria isolated and identified were followed up. A detailed clinical features, demographic data: Malay-Chinese-Indian- others, age, gender were collected from different words of the hospital where they had been admitted. In addition, species of ESBL bacteria identified from each patient was demonstrated.

RESULTS AND DISCUSSION

A total of 34 of drug resistance bacteria were identified from 2502 samples, out of which 25(73.7%) were *K. pneumoniae*, 4 (11.7%) were other *Klebsiella* and 3(8.8%) of the isolates were *Escherichia coli*.

PCR product analysis showed that most common drug resistance gene was TEM, which was identified in 50% of the isolates (Fig. 1) 11%, was found positive for both TEM and SHV. Next 11% of the isolates expressed only SHV genes (Fig. 2).

The patients were admitted in different wards (Table 1) with the clinical manifestations of different diseases. Some were found to be suffered from multiple diseases; some were with chronic fatal diseases and some were infected with multiple causal agents (Table 3). Demographic data with ages and gender of the patients are presented in (Table 2).

It reveals from the Table 1 that the highest (29.4%) percent of patients had been suffering from ESBL infections admitted in Medical word, next to this was in surgery (14%) and urology (14%) and the lowest in Oncology and Radiology (2.9%). ICU, PICU and Pediatric words the percent of ESBL producing was isolated 8.8% in each case. The highest percent of patients showed the

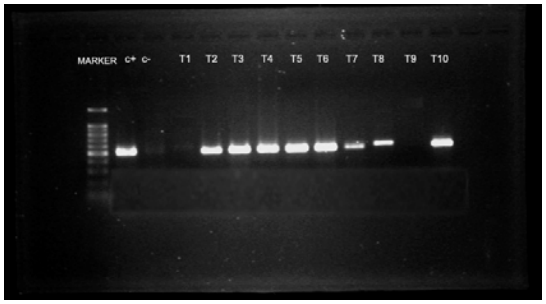

Fig. 1: Detection of drug resistance TEM gene (positive reactions are shown in T2-T8 and T10)

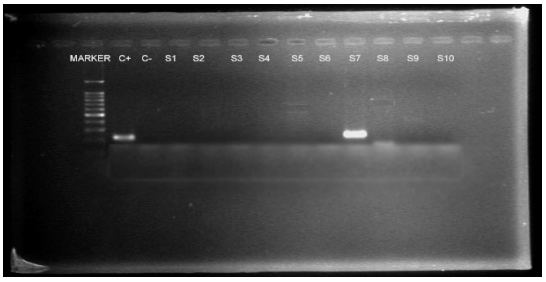

Fig. 2: Detection of drug resistance SHV gene (positive reaction is shown in S7)

Table 1: Distribution of patients according to ward infected with drug resistance Betalactamase producing bacteria

	RACE			
Ward	Malay (%) n = 24	Chinese (%) n = 4	Indian (%) n = 6	Total (%) N = 34
Medical	6 (25.0)	1 (25.0)	3 (50)	10 (29.4)
Surgical	4 (16.7)	1 (25.0)	0	5 (14.7)
Urology	2 (8.3)	1 (25.0)	2 (33.3)	5 (14.7)
ICU	3 (12.5)	0 (0)	0	3 (8.8)
PICU/NICU	2 (8.3)	1 (25.0)	0	3 (8.8)
Paediatric	3 (12.5)	0	0	3 (8.8)
Neurology	2 (8.3)	0	1 (16.7)	3 (8.8)
Oncology	1 (4.2)	0	0	1 (2.9)
Radiology	1 (4.2)	0	0	1 (2.9)

Legends: ICU = Intensive Care Unit, Picu = pediatric Intensive Care Unit, NICU = Neonatal Intensive Care Unit

Table 2: Demographic data of patients infected with drug resistance Betalactamase producing bacteria

	RACE			
	Malay (%) n = 4	Chinese (%) n = 24	Indian (%) n = 6	Total (%) n = 34
Gender				
Male	16(66.7)	2(50)	6(100)	24(70.6)
Female	8(33.3)	2(50)	0	10(29.4)
Age				
≤1 year	3(12.5)	0	0	3(8.8)
2 to 20	3(12.5)	1(25.0)	0	4(11.8)
21 to 45	6(25.0)	1(25.0)	3(50.0)	10(29.4)
>45	12(50.0)	2(50.0)	3(50.0)	17(50.0)

infection of the ESBL bacteria in Medical word might be due to the maximum number of patients' admission in the word.

Table 2 illustrate that 70.6% of the patients identified to have been suffered from ESBL drug resistance bacteria were male and 29.4% were found to identify that as female. The highest number of ESBL bacterial infection was found to the patients those were aged over 45. Decline of the immense response and more chances of infections in aged people made them vulnerable to be infected with these drug resistance bacteria. Moreover, the elderly people that make them susceptible to nosocomical infections often take multiple hospitalizations. Incidence (29.4%) of ESBL bacteria in female patients remained unanswered.

It is observed from the scenario of the clinical features (Table 3) of the patients those had been exposed to ESBL producing drug resistance bacteria that almost all the patients suffered from severe illness and received long term antibiotic therapy. The patients had been suffering from the diseases were: Hodgkin lymphoma, Advance carcinoma of rectum with bilateral hydronephrosis, Pituitary macroadenoma, CVA with recurrent pneumonia, Pneumonia, Cryptococcus meningitis. Acute lymphoblastic leukaemia with line related sepsis, Prematurity 30 W with sepsis, RVD with tuberculosis, Dilated cardiomyopathy, RVD, liver cirrhosis due to Hepatitis C, DM, HPT, Recurrent abscess and Dengue encephalitis. The clinical manifestations and diseases enumerated were almost all are chronic and wasting diseases. It also reveals from the (Table 3) that the isolated ESBL bacteria were : *K. pneumoniae*, *K. terrigena* and *E. coli* out of which the most frequently isolated drug resistance gram negative bacteria was *K. pneumoniae* (25 cases), next *K. terrigena* (4 cases), *K. oxytoca* (2 cases) and *E. coli* (3 cases).

ESBL bacteria stand apart from other strains of bacteria only because they are resistances to some kinds of antibiotics. Otherwise, they do what other bacteria of their species normally do-including causing the same diseases. Disease caused by ESBL organisms is no more acute than the disease caused by another bacterium of the same type. However, due to their resistance to some antibiotics, they can be trickier and more difficult to treat. This leads to longer hospital stays, rising healthcare costs and increased mortality rates. (Jose, 2009). In this study, we identified risk factors for ESBL-producing bacterial colonization among patients admitted in different wards. Commonly identified risk factors identified for ESBL bacteria were-poor functional status, current antimicrobial drug use, chronic renal insufficiency, liver disease, diabetes, cancer etc. These data may be useful for identifying which patients may warrant empiric ESBL-targeted antimicrobial drug therapy.

Clinical features of ESBL producing bacteria and their therapeutic performances were determined in Taiwan by Chiu *et al.* (2005) at Division of Pediatric Infectious Diseases. They observed that the infection-contributed case fatality rate of 3.0% by the ESBL producing bacteria.

A different approach was undertaken by Pitout *et al.* (2004) who performed a population-based laboratory surveillance of hospital and community sites to define the epidemiology of ESBL-producing *E. coli* infections from 157 patients in a large centralized Canadian region during 2000-2002. The incidence was 5.5/100 000 population/year. Seventy-one percent of the patients had community-onset disease and patients 65 years of age and females had significantly higher rates of infection.

Numerous studies have used a case-control design with which to assess risk factors for colonization and infection with ESBL-producing organisms. A common theme among hospitals plague by organisms that produce ESBLs is high volume and indiscriminate use of extended spectrum cephalosporins (Ariffin *et al.*, 2000; Bisson *et al.*, 2002). Specific risk factors observed in our study were length of hospital stay, severity of illness, history of incubation and mechanical ventilation, urinary

Table 3: Clinical features of the patients infected with drug resistance Betalactamase producing bacteria

NO	AGE	SEX	RACE	WARD	DIAGNOSIS	Isolate
1	40	F	C	Medical	DM, HPT, ESRD, pneumonia	700603 *(E.coli)*
2	69	F	C	Surgical	Fracture of right neck of femur	6918 *(K.pneumoniae)*
3	22	M	M	Surgical	Acute myeloid leukaemia	431288 *(E.coli)*
4	55	M	M	Radiotherapy ward	Cervical carcinoma	904816 *(K.pneumoniae)*
5	75	M	I	Urology	DM, HPT, Underlying renal calculi, urosepsis	137227 *(K.pneumoniae)*
6	1	M	M	Paediatric	Intestinal obstruction	754740 *(K.pneumoniae)*
7	56	F	M	Medical	HIV with brain abscess	137630 *(K.pneumoniae)*
8	1	M	C	Paediatric ICU	Liver haemangioma, nosocomial infection with sepsis	41291 *(K.pneumoniae)*
9	64	M	C	Urology	Staghorn calculi with sepsis	751159 *(K.pneumoniae)*
10	50	M	I	Medical	HIV with pneumonia	751789 *(K.pneumoniae)*
11	69	F	M	GICU	Intraabdominal sepsis	751586 *(K.pneumoniae)*
12	22	M	M	Surgical	Hodgkin lymphoma	762630 *(K.pneumoniae)*
13	58	M	M	Urology	Advance carcinoma of rectum with bilateral hydronephrosis	781263 *(K.pneumoniae)*
14	51	M	I	Urology	Urosepsis	795398 *(K.pneumoniae)*
15	39	M	I	Neurology	Pituitary macroadenoma	751984 *(K.pneumoniae)*
16	66	M	M	Medical	CVA with recurrent pneumonia	751537 *(K.pneumoniae)*
17	14	M	M	Paediatric	Pneumonia	215200 *(K. terrigena)*
18	49	M	M	Surgical	RVD with upper GI bleed	712301 *(K.pneumoniae)*
19	1 month	F	M	Paediatric	Hydrocephalus	2351640 *(K.pneumoniae)*
20	51	F	M	Medical	Diabetic ketoacidosis with sepsis	67300 *(K.terrigena)*
21	66	M	M	Medical	DM, HPT, CVA	924138 *(K.pneumoniae)*
22	43	M	I	Medical	Pneumonia	2355894 *(K.pneumoniae)*
23	53	M	M	Neurology	Cryptococcus meningitis	113365 *(E.coli)*
24	14	F	M	Oncology	ALL with line related sepsis	46192 *(K.pneumoniae)*
25	D30	F	M	NICU	Prematurity 30W with sepsis	39944 *(K.oxytoca)*
26	43	M	M	Medical	DM, HPT, ESRD	317193 *(K.oxytoca)*
27	67	M	M	Urology	Urosepsis, underlying staghorn calculi	3804 *(K.pneumoniae)*
28	23	F	M	Neurology	Meningitis	127116 *(K.pneumoniae)*
29	D20	M	M	NICU	Prematurity 28w with sepsis	34499 *(K.terrigena)*
30	43	M	M	Medical	RVD with tuberculosis	436339 *(K.pneumoniae)*
31	37	M	M	GICU	Dilated cardiomyopathy	433879 *(K.pneumoniae)*
32	43	M	I	Medical	RVD, Liver cirrhosis due to Hepatitis C	445220 *(K. Pneumoniae)*
33	62	M	M	Surgical	DM, HPT, Recurrent abscess	451103 *(K. pneumoniae)*
34	23	F	M	GICU	Dengue encephalitis	106521 *(K. terrigena)*

Legends: M: Male; F: Female; Mly: Malay; C: Chinese; I: Indian; DM: Diabetes mellitus; HPT: Hypertension; UTI: Urinary tract infection; ALL: Acute lymphoblastic leukaemia; ESRD: End stage renal disease; RVD: Retro viral disease; GIT: Gastro intestinal disease

or arterial catheterization and previous exposure to antibiotics. Lautenbach and Metlay (2001) in a study observed that use of a variety of other antibiotic classes have been found to be associated with subsequent infections due to ESBL-producing organisms.

Many hospitals have experienced outbreak of ESBL-producing organisms. These outbreaks are often fueled by the transfer of patient between units and between hospitals (Lucet *et al*., 1999). It was found that barrier precautions were often difficult to imply in mobile patient population. Eventually, many of the outbreaks were successfully managed using proper infection control, restriction of the use of oxyimino-cephalosporins and antibiotic cycling (John and Rice, 2000). A successful step in controlling the spread of ESBL-producing organisms involved switching to different classes of broad-spectrum antibiotics for serious infections. The two most successful replacement antibiotics have been carbepenem group and piperacillin-tazobactam (Pena *et al*., 1997). In early outbreaks of ESBL-producing strains were caused by isolates that produced only as single β-lactamase. More recent infection has been caused by organisms with multiple β-lactamases (Bradford *et al*., 1994).

Brook (2009) described the role of betalactamase producing bacteria in mixed infections. He mentioned that betalactamase producing bacteria can play an important role in polymicrobial infections and can have direct pathogenic impact in causing the infections as well as an indirect effect through their ability to produce the enzyme betalactamase. He also pointed out that these bacteria not only survive penicillin therapy but can also protect other penicillin susceptible bacteria by releasing the free enzyme into the environment Finally he concluded that the role of the organisms is the increased failure of antibiotic therapy that causes failure for effective treatment of infectious diseases.

Jorgensen (2008) made a comprehensive discussion on Extended-spectrum beta-lactamase producing organisms. The author mentioned that the bacteria are an increasing challenge for healthcare practitioners fighting healthcare-associated infections. *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* are the most common ESBL-producing pathogens.

The result mentioned that ESBL-producing organisms are generally resistant to many classes of antibiotics and are associated with increased mortality and are difficult to detect and treat. The result commented that widespread use of extended-spectrum, third-generation cephalosporins, is believed to be a major contributor to the emergence of ESBL-producing organisms. The author finally advised that all laboratories related to the identification of bacteria should check for the presence of ESBLs, something they don't always do currently.

A limitation of the present study was is that we did not have access to records of the antimicrobial drugs that patients may have received as outpatients before their hospital admission.

In the present study we could identify the genes of drug resistance betaclamase producing bacteria and clinical features of the patients from where the drug resistance bacteria were identified. The clinical features and patients history described in the study would provide guidance to the clinicians to identify the patients in treating drug resistant bacterial infections. However, because of the enzymes' ability to fight off antibiotics, people with weak immune systems those are at risk such as children, the elderly and people with other illnesses should not be kept in prolonged hospitalization.

Finally, better infection control and hygiene in hospitals, plus controlled and prudent use of antibiotics, is required to minimise the impact of ESBL and the spread of infections.

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