Plasmid and Total Protein Analyses of Extended Spectrum Beta Lactamase Producing Bacteria from *Periplaneta americana* (Coackroaches)

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Abstract

BACKGROUND

The importance of plasmid-encoded Extended Spectrum Beta Lactamase (ESBL) organisms in the dissemination of multi-drug resistance and complicated infections are contributory factors to treatment failure. Despite these, there is a paucity of information regarding plasmid-borne associated infection from primary sources including cockroaches while studies correlating total protein and plasmid-encoded ESBL are also limited especially in cockroaches. The objective of this study was therefore aimed at determining the presence of plasmid in the ESBL organisms from cockroaches as well as their total protein profiles.

MATERIALS AND METHODS

Bacterial isolation, characterization and molecular identification were carried out in our previous work following standard recommended techniques. These molecularly identified organisms which were delineated into twenty-two (22) were further subjected to plasmid and total protein analyses using alkaline lysis and Sodium Dodecyl Sulfate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) respectively.

RESULTS

Results obtained identifies 18(81.8%) of the ESBL isolates to be harbouring plasmids of different molecular weights (2-21.5kbp). The total protein profile of the plasmid-encoded ESBL and the non-plasmid encoded ESBL reveals differential protein expression patterns except for two non-plasmid encoded ESBL isolates that have similar patterns as that of plasmid-encoded ESBL organisms (isolates 2 and 3).

CONCLUSION

There is a need to monitor the primary sources of infection in the epidemiological distribution of resistance. Improving environmental sanitation through proper disinfection of carrier insects may be a means to curtailing such spread.

***Keywords**: Plasmid, Protein, ESBL, Cockroaches, Diversity, Resistance.*

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Introduction

A significant increase in the incidence of infections associated with extended-spectrum beta-lactamase (ESBL) producing organisms has been observed throughout the world (Gupta, 2007; Fatemeh *et al.,* 2012; Abhijit *et al.,* 2013; Majda *et al.,* 2013; Meeta *et al.,* 2013 and Rupinder *et al.,* 2013). These types of organisms have different alleles such as *SHV*-1 (named after the Sulfhydryl-variable active site), *CTX-M*

(which preferentially hydrolyze cefotaxime over ceftazidime and also cefepime with high efficiency), *TEM*-1 (designated *Temoniera*) and *blaCTX-M* (which are well represented in many different genera of the family *Enterobacteriaceae* and *Pseudomonas aeruginosa*). In clinical strains, so many ESBL-encoding genes are located on plasmids of varying sizes (Gonullu *et al.*, 2008).

These organisms have universal dissemination and are readily recovered from various samples including both living (Fatemeh *et al.*, 2012; Abhijit *et al.*, 2013; Popoola *et al.*,2019) and non-living samples (Thomas *et al.*,2015). Their presence in almost every conceivable environment is well reported (Fatemeh *et al.*, 2012; Abhijit *et al.*, 2013; Popoola *et al.*,2019), while some recovery from toilet seats, sinks and toilet environments have also been documented (Balogun *et al.*, 2020).

The potential for direct ingestion of these organisms from street-vended foods has also been represented in some literature (FAO, 2016; Zurita *et al.*, 2019). Several ESBL-producing organisms have been characterized in different foods including fish products (Franzetti *et al.*, 2001), raw and processed milk (Wiedmann *et al.*, 2000; Dogan and Boor, 2003) and among other foods.

Recently, the possibility of insects acting as a mechanical vector for the transmission of antibiotic-resistant bacteria was documented (Rahuma *et al.*, 2005; Popoola *et al.*, 2019) and some of these insects are edible while a few others are microbiologically hazardous upon consumption (Abu-Ghannam and Crowley, 2006). Cockroaches, which are dorso-ventrally flattened insects are known for being abundantly notorious and obnoxious non-biting pests (Mba and Kelly, 2003) and have been reported to be on this planet Earth for more than three hundred million years (Zurek and Schal, 2004). These insects are also known to be well-adapted and include about 4500 species with *Periplaneta americana* and *Blatella germanica* being the most common species (Uneke, 2007).

Their nocturnal and filthy habits make them the ideal carriers of various pathogenic microorganisms (Pai *et al*., 2005; Blazar *et al*., 2011), including *Staphylococcus aureus*, *Streptococcus spp*., *Enterobacteriaceae*, *Pseudomonas aeruginosa* among other bacteria (Pai *et al*., 2005; Fakoorziba *et al*., 2010; Brown and Alhassan, 2015). The role of these insects in the epidemiology of different infections has been reported (Pai *et al*., 2005; Fakoorziba *et al*., 2010).

In a report by Czajka *et al*. (2003), an outbreak of nosocomial disease due to extended-spectrum β-lactamase-producing *K.pneumoniae* in the neonatal unit was attributed to an infestation of the neonatal wards with cockroaches and the continuous presence of these cockroaches in every home are mainly due to complexity of building structures, furniture as well as the emergence of insecticide-resistant strains (Zurek and Gorham, 2008).

Due to the recognition of the increasing importance of ESBL organisms in difficult-to-treat infections and considering the deleterious effect of the associated infection, there is a need to search for more primary sources of ESBL organisms other than in clinical settings to avert their increasing occurrences in human. This study was therefore undertaken to ascertain their mechanisms of resistance beyond ESBL production. This is important because infection with such organisms poses a significant threat to treatment which may subsequently result in death, especially in immune-compromised patients.

Materials and Methods

Study area and sample source

The research was carried out in Ago-Iwoye, Ogun State, Nigeria. Twenty-two (22) molecularly identified ESBL-producing organisms isolated in our previous study

(Popoola *et al.*, 2019) were used for this research. These isolates' source, time of isolation and other necessary parameters are documented in Table 1.

Culturing and subculturing

The isolates preserved on nutrient agar were grown in nutrient agar and subcultured on MacConkey agar under aseptic conditions at 37°C for 24 hours. The isolates were then subcultured into the nutrient broth before agitation in the shaker at 150 rpm at 25°C for 24 hours to obtain the proteins.

Separating bacterial colonies, cell disruption and preparation of crude extracts

Bacterial colonies were separated from the medium by using Whatman number 1 filter paper through a funnel under a sterile condition using sterile PBS in a three stages manner for washing (Bradford, 1976).

Disruption was performed first by grinding in liquid nitrogen and subsequently using glass beads (diameter, 1mm) on a vortex mixture for 1 min until about 80-90% of cells were disrupted. After cell disruption, the crude extracts were separated from intact cells and cell walls remaining by centrifugation at 25000 rpm for 30 min through three stages.

The protein content of these solutions was determined according to the method of Bradford (Latge and Paris, 1991). The supernatants were kept in microtubes at-20°C until used (Laemmli, 1970; Bradford, 1976).

Table 1:
Source of ESBL-producing Gram-negative bacteria used in this study

S/N	Strains	Origin	Part of Samples	Place/Country	Year of Isolation
1	KL11	Ijebu Ode	External surfaces	Ago Iwoye/Nigeria	13/2/2017
2	SM7	Ago sec road	External surfaces	Ago Iwoye/Nigeria	7/2/2017
3	PV3	Martins Kuye	External surfaces	Ago Iwoye/Nigeria	7/2/2017
4	SM8	Omoedumare	External surfaces	Ago Iwoye/Nigeria	7/2/2017
5	EC6	Imere	External surfaces	Ago Iwoye/Nigeria	7/2/2017
6	PV2	Olopomerin	Gut	Ago Iwoye/Nigeria	7/2/2017
7	EC14	Itamerin	External surfaces	Ago Iwoye/Nigeria	31/1/2017
8	PV6	Onabamiro	External surfaces	Ago Iwoye/Nigeria	24/1/2017
9	KL2	Old garage	External surfaces	Ago Iwoye/Nigeria	24/1/2017
10	EC13	Iyalaje	External surfaces	Ago Iwoye/Nigeria	13/12/2016
11	KL1	Aborisade	Gut	Ago Iwoye/Nigeria	29/11/2016
12	EC12	OOUMC	External surfaces	Ago Iwoye/Nigeria	29/11/2016
13	EC3	Fibigbade	External surfaces	Ago Iwoye/Nigeria	29/11/2016
14	EC2	OOUPS	External surfaces	Ago Iwoye/Nigeria	29/11/2016
15	SM5	Konigba	External surfaces	Ago Iwoye/Nigeria	29/11/2016
16	EC9	Imoran	External surfaces	Ago Iwoye/Nigeria	21/11/2016
17	KL9	Ololo	Gut	Ago Iwoye/Nigeria	21/11/2016
18	EC5	Idode	External surfaces	Ago Iwoye/Nigeria	14/11/2016
19	EC4	Koroko	External surfaces	Ago Iwoye/Nigeria	14/11/2016
20	SM3	Ayegbami	External surfaces	Ago Iwoye/Nigeria	14/11/2016
21	KL10	Girisayo	Gut	Ago Iwoye/Nigeria	8/11/2016
22	EC1	Isamuro	External surfaces	Ago Iwoye/Nigeria	8/11/2016

Key: EC-*Escherichia coli;* KL1-*Klebsiella quasipneumoniae;* KL2-*Klebsiella rhinoscleromatis;* PV-*Proteus vulgaris;* SM-*Serratia marcescens;* KL9- *Klebsiella rhinoscleromatis,* KL10-*Klebsiella rhinoscleromatis* KL11-*Klebsiella quasipneumoniae*

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The extracts of bacteria were analysed by (SDS-PAGE) method with 11% separating gel and 4% stacking gel in a discontinuous buffer system according to the method of Laemmli (1970). The extracts were boiled for 5 min with a reducing sample buffer (containing 2-mercaptoethanol) and 35 microlitres of each sample w**ere** loaded on a gel.

Along with the samples, the standard marker (Fermentase) was also electrophoresis, which is a mixture (Staining with Coomassie brilliant blue (G250)**.** Staining was done by using Coomassie brilliant blue G250 (sigma) (Burnie, 1989)

Plasmid isolation

Plasmid extraction of 22 isolates was carried out using the alkaline lysis method of Takahashi and Nagano (Takahashi and Nagano, 1984). Plasmid DNA bands were detected by electrophoresis on 0.8% horizontal agarose gel pre-stained with ethidium bromide (0.5µg/mL) and visualized under UV light. The sizes of the plasmid DNA bands were determined by extrapolation based on the mobilities of Hind III digested λ DNA co-electrophoresed with the plasmid DNA samples (Meyers *et al*., 1976).

Results

Plate 1 and 2 depicts the presence of plasmids of different molecular weights (2-21.5kbp) in the ESBL-producing organisms.

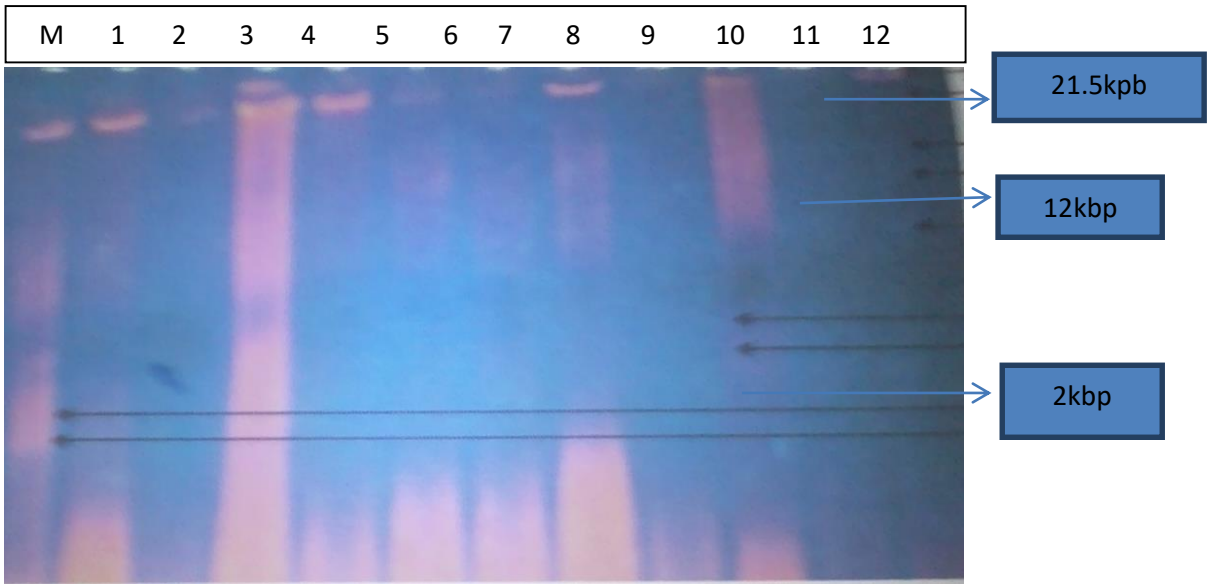

***Figure 1*:**
Plate 1 Plasmid profile of ESBL-producing organisms

Key: 1- *Escherichia coli* (EC1), 2-*Klebsiella quasipneumoniae* (KL1), 3- *Escherichia coli* (EC3), 4- *Proteus vulgaris*(PV6), 5- *Serratia marcenscens*(SM8), 6- *Klebsiella rhinoscleromatis*(KL9), 7- *Serratia marcenscens*(SM7), 8- *Klebsiella rhinoscleromatis* (KL10), 9- *Escherichia coli* (EC12), 10- *Escherichia coli* (EC9), 11-*Escherichia coli* (EC5)

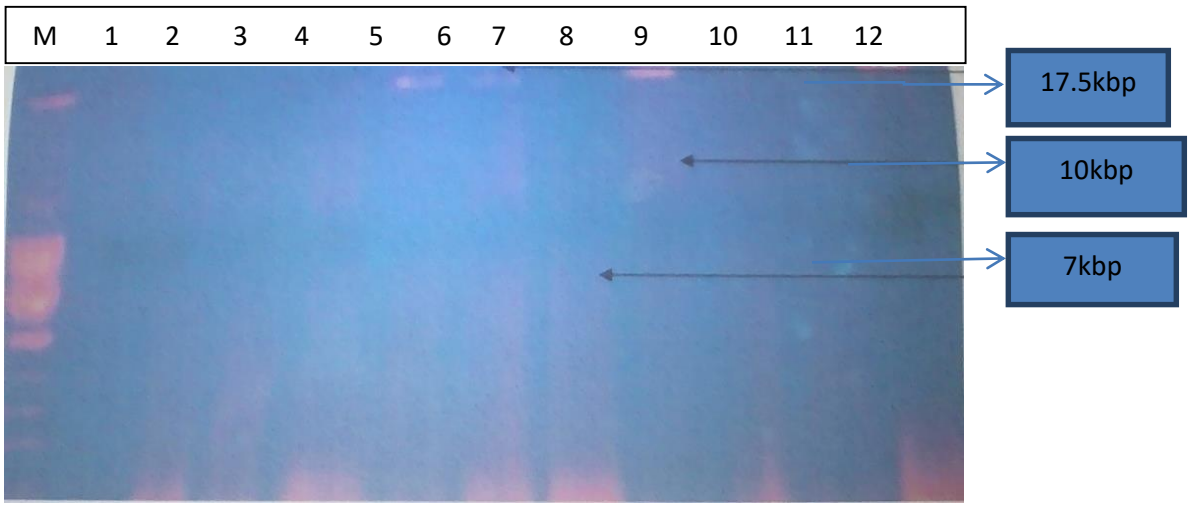

Figure 2:

Plate 2: Plasmid profile of ESBL-producing organisms

Key: 1- *Escherichia coli* (EC6), 2-*Klebsiella rhinoscleromatis* (KL2), 3- *Klebsiella rhinoscleromatis* (KL11) 4- *Proteus vulgaris* (PV3), 5- *Serratia marscencens*(SM13), 6- *Proteus vulgaris*(PV2), 7- *Serratia marscencens*(SM3), 8- *Serratia marscencens*(SM5), 9- Escherichia *coli* (EC14), 10- *Escherichia coli* (EC2), 11-*Escherichia coli* (EC4)

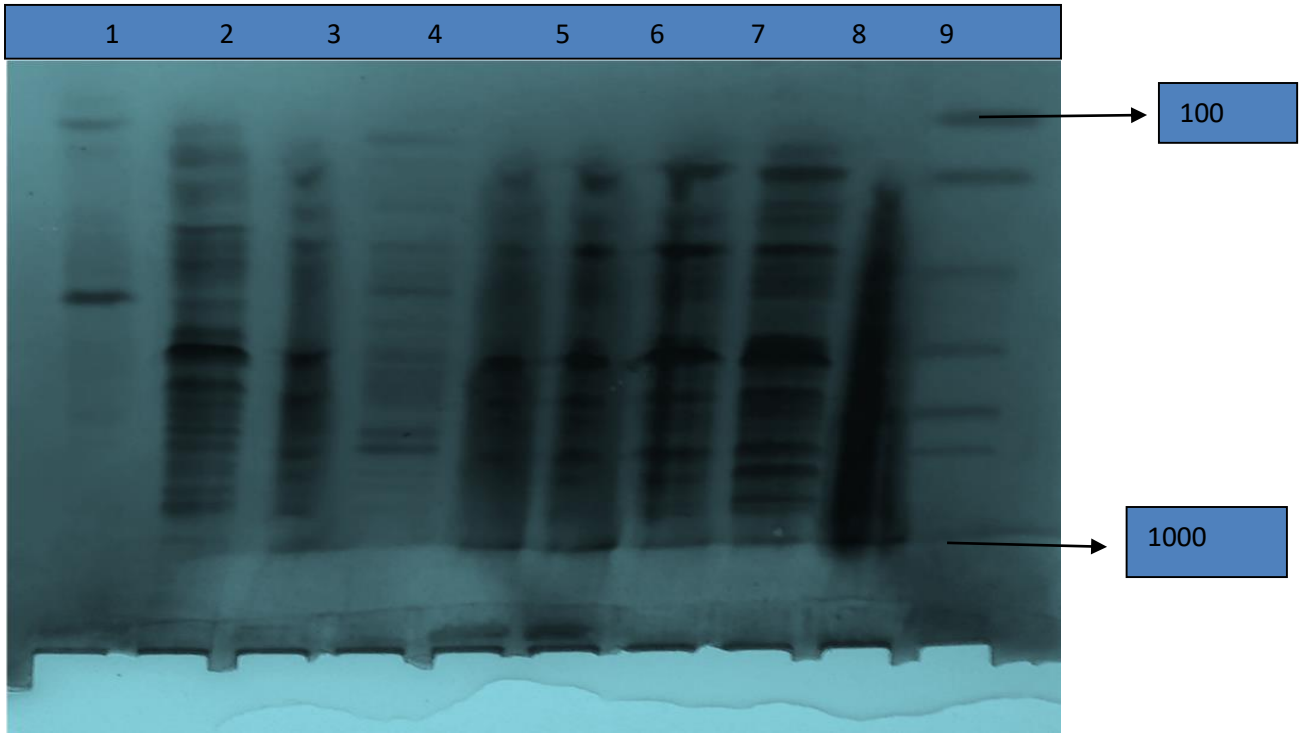

Figure 3:

Plate 3: Outer membrane proteins of ESBL and non-ESBL producing Gram-negative bacteria

Key: non-ESBL isolates (1-, 2-, 3-, 4-), ESBL isolates (5- *Escherichia coli*, 6- *Serratia marcescens*, 7- *Klebsiella rhinoscleromatis*, 8- *Proteus vulgaris*), 9- control, M- DNA Marker (100bp)

A few of these organisms were also found not harbouring plasmids of any size. The total proteins of ESBL and non-ESBL Gram-negative bacteria typed using SDS-PAGE analysis connotes that the isolates (ESBL and non-ESBL isolates) expressed different outer membrane proteins as shown by their banding patterns. Even though isolates two and three which are non-ESBL producers had similar outer membrane protein expression patterns as the ESBL isolates (Plate 3).

Discussion

The elevated trend of multi-drug resistance to the different antibiotics tested observed in this study is a serious challenge that has negative consequences on infection control and hospital management of patients (Velez and Sloand, 2016; Chandran *et al*., 2008). This resistance was found to be higher in *Klebsiella* spp. followed by *Escherichia coli* and *Shigella flexneri* respectively. Our finding is analogous to that documented by Reinert *et al*. (2007), who documented high levels of resistance to some of the most commonly prescribed antibiotics including extended-spectrum cephalosporins, carbapenems and fluoroquinolones. The European Antibiotic Resistance Surveillance System report (2008) further buttresses the continuous increase in resistance to third-generation cephalosporins by both *Escherichia coli* and *Klebsiella* spp. The resistance of these organisms to third-generation cephalosporins may be due to the production of extended-spectrum beta-lactamases which are known for hydrolyzing these antibiotics (Rawat and Nair, 2010). Some of the non-ESBL-producing isolates also showed some form of resistance to the third-generation antibiotics and this may be attributed in part to the lack of permeation of porins and AmpC beta-lactamase production (Mohamudha *et al*., 2010) among other factors.

The fact that the majority of the ESBL-producing organisms expressed different outer membrane proteins from the Non ESBL organisms is an indication that their antigenicity does not follow the same electrophoretic patterns and this further affirms the possibility of using polyacrylamide gel electrophoresis for typing such organisms. However, the use of a probe to check for a possible cross-reaction within and between isolates has been suggested (Thomas *et al*., 2015).

The isolation of plasmid of various sizes affirmed in some of the bacterial isolates is corroborating the findings of Lincopan *et al*. (2006) who reported that the increased secretion of extended-spectrum beta-lactamases enzymes is mostly mediated by the presence of plasmid in such bacteria. This type of resistance is now observed in almost all species of *Enterobacteriaceae* and is currently disseminated throughout the world thus resulting in a very high diversity of plasmid.

In Nigeria, Thomas *et al*. (2015) reported the plasmid profile of some ESBL organisms and affirmed the diversity of plasmids of various sizes with 32(43.2%) harbouring one plasmid, 37(50%) harbouring two genes while only five carried three plasmids each. This observation also corroborates the finding that most of the isolates harboured not less than three different sizes of the plasmid. The subsequent treatment of all the plasmid-carrying bacteria with Acridine orange resulted in a loss of resistance by these isolates. This may be related to the permeability through the outer membrane and to the location of antibiotic-resistant genes carried on different plasmids and such ability further eliminates R plasmids (Carattoli, 2009). Generally, the low copy number of plasmids is easily cured compared to a plasmid of higher copy numbers, however, research suggested differences in DNA polymerase and RNA.

In conclusion, results obtained from our study have shown that most of the ESBL-producing Gram-negative bacteria isolated from

cockroaches are plasmid-borne and mostly expressed differential protein patterns from the non-plasmid encoded organisms to infer probable genetic variability.

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