Tanzania Journal of Science 48(3): 633-639, 2022
ISSN 0856-1761, e-ISSN 2507-7961

Pulse-Field Analysis Diversity of Extended Spectrum Beta Lactamases Producing Gram Negative Bacteria Isolated from *Periplaneta americana* (Cockroaches)

Omolara D. Popoola, Moses O. Efuntoye, Georgia C. Agu and Benjamin T. Thomas*
Department of Microbiology, Olabisi Onabanjo University, Ago Iwoye, Ogun State, Nigeria
**Corresponding author, e-mail: benjamin.thomas@oouagoiwoye.edu.ng*
Co-authors' e-mails: omolara.popoola@oouagoiwoye.edu.ng, efuntoye.olusola@oouagoiwoye.edu.ng, agu.georgia@oouagoiwoye.edu.ng
Received 6 Jun 2022, Revised 20 Jul 2022, Accepted 15 Aug 2022, Published Sep 2022
DOI: https://dx.doi.org/10.4314/tjs.v48i3.10

Abstract

Extended spectrum beta lactamase (ESBL) producing bacteria are organisms of public health significance that have been implicated in prolonged hospital stay, treatment failures and higher fatalities. These organisms were originally thought to be widely distributed in hospital, but have since grown in terms of their epidemiological coverage. This study was therefore aimed at using pulse-field gel electrophoresis (PFGE) to understand the diversity of ESBL producing organisms originating from cockroaches in Ago-Iwoye, Nigeria. The diversity was determined by using a set of twenty six (26) ESBL producing bacterial strains isolated from cockroaches sampled in Ago-Iwoye community, Ogun State, Nigeria. PFGE analysis revealed three pulsotypes (distinct profile) with no distinct isolates present on a pulsotype. It was however observed that no pulsotype had less than two (2) isolates in any case, while isolates from different communities displayed identical profiles. In conclusion, clonal distributions of ESBL producing organisms on cockroaches are without bias to specimens and/or the community sampled.

Keywords: ESBL, Gram negative bacteria, PFGE, Diversity.

Introduction

The diversity of microorganisms in every sphere of life ranging from agriculture through medicine and biotechnology to the environment has been documented (Thomas et al. 2012a, 2012b, 2012c, Kolawole et al. 2013, Osho et al. 2013, Thomas et al. 2017, Agu et al. 2018). These microorganisms including the Gram negative bacteria have been indicted in several infectious diseases such as septicemia, urinary tract infections, wound infections, cerebro-spinal meningitis among other infections (Donnerberg 2009) and their significance could be further exacerbated by their ability to be transferred by animate objects including houseflies (Popoola et al. 2019a), cockroaches (Popoola et al. 2019b) among other insects, thereby further widening their epidemiology. What is more deleterious is when these so called Gram negative bacteria produce ESBL which are now well known for their hydrolyzing activities on the extended spectrum cephalosporin (Lakshmi et al. 2014). These enzymes have significantly hindered several treatment successes, thus depicting an apparent upsurge in the global incidence of ESBL associated infections (Gupta 2007, Thomas et al. 2012d, Awari et al. 2013, Kritu et al. 2013, Majda et al. 2013, Meeta et al. 2013, Rupinder et al. 2013).

Presently, the major growing concern is the ubiquitous dissemination of different types of ESBL alleles (Day et al. 2016),

which are well known for their promiscuity especially between themselves. One of the negative reports on these organisms was the outbreak of nosocomial infections in a neonatal ward due to ESBL producing *Klebsiella pneumoniae*, and despite this, the epidemiology of ESBL producing bacteria is still very difficult to understand because of its complexity and increasing boundaries between hospital and the community (Shaikh et al. 2014).

Consequently, these organisms have a global distribution (Babini and Livermore 2000, Rupp and Fey 2003), but just like Hawksworth (2001) noted that, less is known about the variations of fungal diversity and composition along different gradients such as latitude, altitude, productivity and salinity, we also observed that little is known about pulse-field analysis of diversity of ESBL producing Gram negative bacteria isolated from cockroaches in Ago Iwoye, Ogun State, Nigeria. We however observed that even where diversity studies on these organisms were carried out, attentions were more on clinical isolates than those stemming from insects and other animate objects. It is however not impossible to find work-done elsewhere using traditional methods, but as early as 1975, the importance of using sound techniques and statistics for biodiversity studies have been stressed (May 1975). This is important in order not to reduce the fine resolution required to score diversity (Thomas et al. 2017). This study, was therefore, aimed at determining the diversity of ESBL producing organisms using pulse-field gel electrophoresis technique.

Materials and Methods

Bacterial strains

A set of 40 Gram negative bacteria was used in this study, from which a subset of 26 ESBL producing strains characterized by a primer specific polymerase chain reaction were selected. The characteristics of these isolates in terms of origin, time of isolation and place of isolation are depicted in Table 1.

DNA isolation, amplification and sequencing of ESBL genes

Each specimen was stirred directly into 200 µl sterile saline and extracted using a QIAamp DNA mini kit (Qiagen) according to a protocol adapted for extraction of DNA from bacterial cells, as described by Fredricks et al. (2005). In brief, each sample was pre-incubated at 99 °C for 20 min and then processed as suggested by the manufacturer. After the addition of the cellular lysis buffer, the sample was incubated again at 99 °C for 10 min. The supernatant, a template DNA in the PCR reaction was transferred into a new pre-labelled eppendorf tube by gentle aspiration using a micropipette (Kiratisin et al. 2008). PCR amplification of *TEM*, *SHV* and *CTX-M* genes were performed as described previously using their specific primers (Table 2). For amplification, 2 µl of template DNA was added to 18 µl mixture containing 4.0 µl Master mix (dNTPs, taq polymerase, buffer, $MgCl_2$), 0.2 µl of primer pair and 13.6 µl of sterile water). The reaction was performed in Simpliamp PCR system thermo cycler (Applied Biosystems, USA) under the following conditions: Initial denaturation at 94 °C for 5 minutes followed by 30 cycles of 30 seconds denaturation at 94 °C, 60 seconds annealing at 58 °C, 90 seconds extension at 72 °C, and a final extension at 72 °C for 5 minutes (Kiratisin et al. 2008). PCR products were detected with ethidium bromide fluorescence using the photo image system (BioRad, UK) after 45 minutes electrophoresis in 1.5% TAE agarose gel done at 100 volts. Positive controls for *TEM*, *SHV* and *CTX-M* were used in every run.

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	EC1	Isamuro	External surfaces	Ago Iwoye/Nigeria	8/11/2016
2	EC10	Fowoseje	External surfaces	Ago Iwoye/Nigeria	8/11/2016
3	KL6	Obaruwa	Gut	Ago Iwoye/Nigeria	8/11/2016
4	KL10	Girisayo	Gut	Ago Iwoye/Nigeria	8/11/2016
5	SM3	Ayegbami	External surfaces	Ago Iwoye/Nigeria	14/11/2016
6	EC4	Koroko	External surfaces	Ago Iwoye/Nigeria	14/11/2016
7	EC8	Igan	External surfaces	Ago Iwoye/Nigeria	14/11/2016
8	EC5	Idode	External surfaces	Ago Iwoye/Nigeria	14/11/2016
9	KL9	Ololo	Gut	Ago Iwoye/Nigeria	21/11/2016
10	EC9	Imoran	External surfaces	Ago Iwoye/Nigeria	21/11/2016
11	SM1	Ijesa road	External surfaces	Ago Iwoye/Nigeria	21/11/2016
12	SM5	Konigba	External surfaces	Ago Iwoye/Nigeria	29/11/2016
13	EC2	OOUPS	External surfaces	Ago Iwoye/Nigeria	29/11/2016
14	EC3	Fibigbade	External surfaces	Ago Iwoye/Nigeria	29/11/2016
15	EC12	OOUMC	External surfaces	Ago Iwoye/Nigeria	29/11/2016
16	KL1	Aborisade	Gut	Ago Iwoye/Nigeria	29/11/2016
17	EC13	Iyalaje	External surfaces	Ago Iwoye/Nigeria	13/12/2016
18	KL2	Old garage	External surfaces	Ago Iwoye/Nigeria	24/1/2017
19	PV6	Onabamiro	External surfaces	Ago Iwoye/Nigeria	24/1/2017
20	EC14	Itamerin	External surfaces	Ago Iwoye/Nigeria	31/1/2017
21	PV2	Olopomerin	Gut	Ago Iwoye/Nigeria	31/1/2017
22	EC6	Imere	External surfaces	Ago Iwoye/Nigeria	7/2/2017
23	SM8	Omoedumare	External surfaces	Ago Iwoye/Nigeria	7/2/2017
24	PV3	Martins Kuye	External surfaces	Ago Iwoye/Nigeria	7/2/2017
25	SM7	Ago Sec road	External surfaces	Ago Iwoye/Nigeria	7/2/2017
26	KL11	Ijebu ode	External surfaces	Ijebu ode/Nigeria	13/2/2017

Key: EC-*Escherichia coli*, KL1-*Klebsiella quasipneumoniae*, KL2-*Klebsiella rhinoscleromatis*, PV-*Proteus vulgaris*, SM-*Serratia marcescens*, KL9- *Klebsiella rhinoscleromatis*, KL6-*Klebsiella quasipneumoniae*, KL10-*Klebsiella rhinoscleromatis*

Table 2: ESBL specific primers

ESBL genes	Length	Sequence (5'–3')
***CTX-M* F**	27 bp	$^{5'}$ATGGTTAAAAAATCACTGGGYCAGTTC$^{3'}$
***CTX-M* R**	30 bp	$^{5'}$TCACAAACCGTYGGTGACGATTTTAGCCGC$^{3'}$
***SHV* F**	21 bp	$^{5'}$ACCTTTAAAGTAGTGCTCTGC$^{3'}$
***SHV* R**	21 bp	$^{5'}$CACCATCCACTGCAGCAGCTG$^{3'}$
***TEM* F**	22 bp	$^{5'}$ACAGCGGTAAGATCCTTGAGAG$^{3'}$
***TEM* R**	21 bp	$^{5'}$GAAGCTAGAGTAAGTAGTTCG$^{3'}$

(Source: Kiratisin et al. 2008).

Pulse-field gel electrophoresis (PFGE)

PFGE was performed according to the Pulse Net protocol of Centre for Disease Control and Prevention Atlanta, USA (CDCP 2018). In brief, the agarose embedded DNA was restricted with *Xba*I (New England Biolabs, England, UK) at 37 °C for 16 hrs. Electrophoresis was performed on 1% Agarose gel (Bio-Rad, UK) 0.5X TBE buffer (Sigma). The PFGE conditions for the selected Gram negative bacteria were 6V, 2.2S-54S pulse, for 20 hrs. Electrophoresis was conducted using CHEF Drive II (Bio-Rad, UK). Strain differentiation by PFGE analysis was achieved by comparison of band patterns. Patterns were normalized on the

basis of the molecular weight marker. The similarity coefficient (SAB) of sample pairs was calculated based on band positions by using the DICE metric. The genetic relationships among isolates were computed by cluster analysis performed on the matrix of genetic similarities. Cluster analysis was performed by means of the unweighted paired group method using arithmetic average (UPGMA) (Hunter and Fraser 1989). Dendograms were generated to visualize relationships among the isolates. The cut-off in the dendograms were calculated at a SAB of 0.99 as a threshold for defining clone of genetically similar isolates and SAB of 0.8 to define cluster of isolates. The discriminatory power of the applied PFGE typing method was assessed by calculating the discriminatory index D based on application of Simpson's index of diversity as described previously (Hunter and Gaston 1988, Hunter and Fraser 1989). Molecular sizes of the bands were also calculated with Gelcompar II (Applied Maths) by using a calibration curve based on a synthetic regression curve derived from the reference bands.

Results

The results of the pulse-field gel electrophoresis of the analyzed ESBL producing organisms revealed that the ESBL organisms were appropriately discriminated into three distinct pulsotypes, viz; pulsotype 1-3 with pulsotype 1 found to be the most abundant having a percentage distribution of 19 (73.1%), while pulsotypes 2 and 3 have 3 (11.5%) and 4 (15.4%) distribution rates, respectively (Plate 1). Of these PFGE discriminated organisms, twenty-one (21) were recovered from the cockroach external surfaces with 17 classified together on the same pulsotype, while two each were found on pulsotypes 2 and 3, respectively. On the other hand, the remaining five (5) organisms were from the gut environment of the cockroach (Table 3). No pulsotype was however found to have less than two (2) isolates in any case.

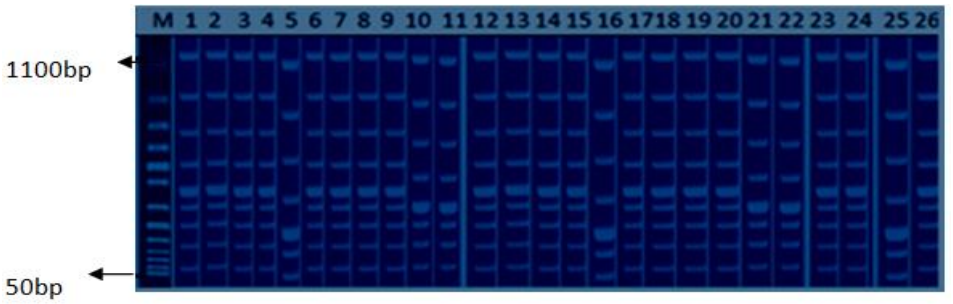

Plate 1: Pulse field gel electrophoresis of the ESBL producing organisms.
Key: 1-26 are appropriately defined in Table 1.

Table 3: Distribution of pulsotypes of ESBL producing Gram negative bacteria according to their origins and parts of samples analyzed

Pulsotypes	Cockroach external surfaces		Cockroach gut environment	
	2016	2017	2016	2017
1	1–2, 6–9, 12–15, 17	18–20, 23–24, 26	3–4	–
2	5	25	15	–
3	10–11	–	21	22

Key: 1-26 = defined in Table 1. (–) = No ESBL producing organism recovered.

Discussion

The extended spectrum beta lactamases producing bacteria are known for hydrolyzing extended spectrum beta lactam antibiotics (Rawat and Nair 2010) and are usually connected with serious challenges that have negative consequences on infection control and clinical management of patients (Chandra et al. 2008, Velez and Sloand 2016), but understanding of their diversity

has been marred by their wide epidemiological coverage among other factors. In this study, the pulse-field gel electrophoresis of extended spectrum beta lactamases producing Gram negative bacteria was studied and it was found that these organisms were discriminated into three pulsotypes. This observation is not unexpected as comparison of PFGE patterns has been extensively used in food epidemiological studies to confirm the sources of disease, in addition to monitoring the geographical and temporal changes in food borne outbreaks caused by bacterial pathogens such as *Listeria monocytogenes, Salmonella species, Vibrio* spp. among other Gram negative bacteria (Goering 2010, Allerberger 2012, Okada et al. 2012).

Not too long ago, the potential of PFGE in discriminating several subgroups inside cereulide producing strains of *Bacillus cereus* using macro restriction analysis was documented (Kim et al. 2011). This observation coupled with our findings is an attestation to the relevancy of this technique in genetic diversity study. The PFGE did not only discriminate the 26 studied isolates into three pulsotypes, but also generated some salient observations including; characterization of isolates from different communities on the same pulsotypes regardless of species. This observation however may be attributed to the possibility of these organisms emanating from a common source or possibility of the same organisms been shared between the different communities (Dudek et al. 2019).

Consequently, pulsotype six has strains from another community (Ijebu ode), in addition to those found in Ago Iwoye. Our analysis also documented pulsotype 1 as the most abundant and geographical distributed pulsotype accounting for approximately 31% of all the collected strains, while none of the pulsotypes were found having less than 7.6% (2) of isolates. The presence of at least two isolates on each pulsotype may not be unconnected to the filthy nature of the experimental insects which are known to wander around dirty environment including feeding and breeding in decaying organic matter (Robinson 2005).

In general, our findings emphasize the importance of PFGE characterization of ESBL Gram negative bacteria. However, in order not to reduce the fine resolution required to score diversity of ESBL organisms, it may be necessary to measure the efficiency of this technique relative to others including genomic multilocus sequence typing (Enright et al. 2002), especially considering the rapid advances of whole genome sequencing and the plethora of growing genomic data-bases (Van der Auwera et al. 2013). It is noteworthy to affirm that understanding the true genetic diversity of extended spectrum beta lactamases producing Gram negative bacteria is crucial to controlling the ever growing infections attributable to ESBL producing Gram negative bacteria. It is important to now view cockroaches as proxies for environmental and public health surveillance.

References

Agu GC, Thomas BT, Ogunkomaya AM and Umeh SO 2018 Beta lactamase producing *Staphyloccoccus aureus* isolated from some meat and meat based foods. *Afr. J. Sci. Nature* 7: 66-75.

Allerberger F 2012 Molecular typing in public health laboratories: from an academic indulgence to an infection control imperative. *J. Prev. Med. Public Health* 45: 1-7.

Awari A, Nighute S and Khatoon M 2013 Study of urinary isolates with reference to extended spectrum beta lactamases detection and antibiogram. *J. Evol. Med. Dent. Sci.* 2(9): 1049-1056.

Babini GS and Livermore DM 2000 Antimicrobial resistance amongst *Klebsiella spp*. collected from intensive care unit in Southern and Western Europe in 1997–1998. *J. Antimicrob. Chemother.* 45: 183-189.

Centre for Disease Control and Prevention (CDCP) 2018 Pulse-field gel electrophoresis (PFGE) retrieved from http://www.cdc.gov/pulsenet/pathology on 17th May 2019.

Chandran A, Hatha AAM, Varghese S and Sheeja KM 2008 Prevalence of Multiple Drug Resistant *Escherichia coli* serotypes in a Tropical Estuary, India. *Microbes Environ*. 23(2): 153-158.

Day MJ, Rodríguez I, van Essen-Zandbergen A, Dierikx C, Kadlec K, Schink AK, Wu G, Chattaway MA, DoNascimento V, Wain J and Helmuth R 2016 Diversity of STs, plasmids and ESBL genes among *Escherichia coli* from humans, animals and food in Germany, the Netherlands and the UK. *J. Antimicrob. Chemother*. 71(5): 1178-1182.

Donnerberg MS 2009 Enterobacteriaceae. In: Mandell GL, Douglas RG, Bennett JE, Dolin R (Eds) Principles and Practice of Infectious Diseases. 7th ed. New York. Elsevier, Churchill Livingstone. p. 2815-2833.

Dudek B Książczyk M Krzyżewska, E, Rogala K, Kuczkowski M, Wozniak-Biel A, Korzekwa K, Korzenioswka-Kowal A, Ratajzczak R, Wieliczko A, Rybka J and Bugla-Ploskonska G 2019 Comparison of the phylogenetic analysis of PFGE profiles and the characteristic of virulence genes in clinical and reptile associated Salmonella strains. *BMC Vet. Res*. 15: 312.

Enright MC, Robinson DA Randle G, Feil EJ, Grundmann H and Spratt BG 2002 The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *Proc. Natl. Acad. Sci.* 99: 7687-7692

Fredricks D, Smith C and Meier A 2005 Comparison of six DNA extraction methods forrecovery of fungal DNA as assessed by quantitative PCR. *J. Clin. Microbiol*. 43(10): 5122-5128.

Goering RV 2010 Pulsed field electrophoresis: a review of application and interpretation in molecular epidemiology of infectious disease. *Infect. Genet. Evol*. 10: 866-875.

Gupta V 2007 An update on newer β-lactamases. *Indian J. Med. Res*. 126(5): 417-427.

Hawksworth DL 2001 The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycol. Res*. 105: 1422-1432.

Hunter PR and Gaston MA 1988 Numerical index of the discriminatory ability of typing systems: an application of Simpson Index of diversity. *J. Clin. Microbiol.* 26: 2465-2466.

Hunter PR and Fraser CAM 1989 Application of a numerical index of discriminatory power to a comparison of four physiochemical typing methods for *Candida albicans*. *J. Clin. Microbiol.* 27: 2156-2160.

Kim JB, Park JS Kim MS, Hong SC Park JH and Oh DH 2011 Genetic diversity of emetic toxin producing *Bacillus cereus* Korean Strains. *Int. J. Food Microbiol*. 150: 66-72.

Kiratisin P Apisarnthanarak A Laesripa C and Saifon P 2008 molecular characterization and epidemiology of extended-spectrum-lactamase- producing *Escherichia coli* and *Klebsiella pneumonia* isolates causing health care-Associated infection in Thailand, where the CTX-M family is endemic. *Antimicrob. Agents Chemother.* 52: 2818-2824.

Kolawole RM, Thomas BT Adekunle AA and Oluwadun 2013 Postharvest pathogenic fungi of wheat circulating in Lagos State, Nigeria. *Am. J. Res. Commun.* 1(12): 421-428.

Kritu P, Prakash G, Shiba KR, Reena KM, Ram NS and Ganesh R 2013 Antibiogram typing of Gram negative isolates in different clinical samples of a tertiary hospital. *Asian J. Pharm. Clin. Res.* 6(1): 153-156.

Lakshmi R, Nusrin KS, Ann GS, Sreelakshi KS 2014 Role of beta lactamases in antibiotic resistance: a review. *Int. Res. J. Pharm.* 5: 37-40.

Majda Q Najma A and Summyia B 2013 Evaluation of extended spectrum beta-lactamase mediated resistance in *Escherichia coli* and *Klebsiella* in urinary tract infection at a tertiary care hospital. *Biomedica* 29: 78-81.

May RM 1975 Patterns of species abundance and diversity. In: Cody ML and Diamond

JM (Eds) Ecology and Evolution of Communities, 81-120. Cambridge, MA: Belknap Press of Harvard University Press.

Meeta S, Sati P and Preeti S 2013 Prevalence and antibiogram of extended spectrum β-lactamase (ESBL) producing Gram negative bacilli and further molecular characterization of ESBL producing *Escherichia coli* and *Klebsiella* spp. *J. Clin. Diag. Res*. 7(10): 2168-2172.

Okada K, Roobthaisong A, Nakagawa I, Hamada S and Chantaroj S 2012 Genotypic and PFGE/MLVA analyses of *Vibrio cholerae* O1: geographical spread and temporal changes during the 2007-2010 cholera outbreaks in Thailand. *PLoS One* 7: e30863.

Osho A, Thomas BT, Akande YA and Udor RD 2013 Toothbrushes as formites. *J. Dent. Oral Hyg*. 5(9): 92-94.

Popoola OD, Thomas BT, Aina SA and Sabitu AN 2019a *Musca domestica* as a vector for the transmission of multidrug resistant *Salmonella* and *Shigella* species. *Nigerian J. Sci. Res.* 18(1): 38-41.

Popoola OD, Thomas BT and Efuntoye MO 2019b A comparative study of cultural and molecular techniques for the identification of bacterial contaminants of cockroaches (*Periplaneta americana*). *Afr. J. Cell. Pathol.* 11(3): 17-22.

Rawat D and Nair D 2010 Extended-spectrum b-lactamases in Gram negative bacteria. *J. Glob. Infect Dis*. 2(3): 263-274.

Robinson WH 2005 Handbook of Urban Insects and Arachnids. Cambridge University Press. 35-64.

Rupinder B, Geeta W and Shikha J 2013 Prevalence of extended spectrum β-lactamases in multi-drug resistant strains of gram negative *Bacilli. J. Acad. Indus. Res.* 1(9): 558-560.

Rupp M and Fey PD 2003 Extended spectrum–lactamase (ESBL)-producing Enterobacteriaceae. *Drugs* 63(4): 353-365.

Shaikh S, Fatima J, Shakil S, Mohd S, Rizvi D and Kamal MA 2014 Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. *J. Med. Internet Res*. 16(4): 90-101.

Thomas BT, Effedua HI, Davies A and Oluwadun A 2012a Prevalence of antibiotic resistant bacteria in dried cassava powder (garri) circulating in Ogun State, Nigeria. *Academia Arena* 4(1): 9-13.

Thomas BT, Adeleke AJ, Adebayo OO and Musa OS 2012b Survival of *Escherichia coli* 0157:H7 in kunun-zaki at different storage temperatures. *Reseacher* 4(8): 13-16.

Thomas BT, Effedua HI, Popoola OD and Oluwadun A 2012c Growth and survival of gastroenteritis pathogens in dried cassava powder (garri). *New York Sci. J.* 5(2): 9-14.

Thomas BT, Agu GC, Musa OS, Adeyemi MT, Davies OO, Adesoga O and Ogueri OC 2012d Cross class resistance to non beta lactams antimicrobials in extended spectrum beta lactamases producing *Escherichia coli*-a concern to health practitioners. *Int. Res. J. Microbiol.* 3(2): 050-054.

Thomas BT, Ogunkanmi LA, Iwalokun BA and Agu GC 2017 Molecular characterization and strain typing of fungal contaminants of processed *Manihot Esculenta* Crantz (*garri*) in ogun state, Nigeria. *Ann. Health Res.* 3(2): 112-117.

Van der Auwera GAM 2013 From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. *Current Protocols in Bioinformatics* 11.10.1-11.10.33.

Velez R and Sloand E 2016 Combating antibiotic resistance, mitigating future threats and ongoing initiatives. *J. Clin. Nurs*. 25: 1886-1889.