East African Scholars Journal of Medicine and Surgery
Abbreviated Key Title: EAS J Med Surg
ISSN: 2663-1857 (Print) & ISSN: 2663-7332 (Online)
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-6 | Issue-4 | April-2023 | DOI: 10.36349/easjms.2023.v06i04.004

Original Research Article

Molecular Detection of PapC and hlyA Virulence Genes in Escherichia coli from Clinical Isolates in Bayelsa State, Nigeria

Tolulope Olukemi Alade[1*], Chindinma Daokoru-Olukole[2], Oluwasegun Matthew Abidogun[3]

[1]Department of Medical Laboratory Sciences, Faculty of Basic Medical Sciences, College of Health Sciences
[2]Department of Microbiology, Niger Delta University, Bayelsa State, Nigeria

Article History
Received: 06.01.2023
Accepted: 11.02.2023
Published: 28.04.2023

Journal homepage:
https://www.easpublisher.com

Quick Response Code

Abstract: *Escherichia coli*, a Gram-negative rod organism and the commonest urinary pathogen causing 60-90% of Urinary infections. Two virulence genes (Pap C and hlyA) had been reported to be involved in *Escherichia coli* pathogenicity and its resistance to antibiotics. The study aimed at detecting PapC and hlyA in *E. coli* isolated from clinical specimens in two main Tertiary hospitals (Federal Medical Centre and Niger Delta University Teaching Hospital) in Bayelsa State. One hundred and forty-five specimens were collected and analyzed using the standard Bacteriological technique. Antibiotics Susceptibility testing was carried out using the Kirby Bauer method and Polymerase Chain Reaction (PCR) was employed using ABI 970 amplified Biosystem thermal cycler. The susceptibility pattern showed that 18(90%) isolates were resistant to Nalixidic acid, 16(80%) to Ampicillin, 15(75%) to Ceporex, 14(70%) to Septrin and Augmentin, 12(60%) to Reflacine, 11(55%) to Ciprofloxacin, 9(45%) to Gentamycin, 8(40%) to Tarivid and 4(20%) to Streptomycin. Fifteen PCR products were resolved on 1.0% agarose gel, out of which 8(53.3%) harboured Pap C while 1(6.7%) harboured hlyA. Seven of the eight Pap C-carrying isolates were susceptible to Ciprofloxacin while the HlyA virulence gene enhances the resistance of the organism to Ciprofloxacin. Therefore, *the E. coli* Pap C gene may be incorporated into Ciprofloxacin production to curb antibiotic resistance.

Keywords: Pap C, hlyA, Escherichia coli, Clinical isolates and Bayelsa.

INTRODUCTION

Escherichia coli is classified as a rod-shaped, Gram-negative bacterium in the *Enterobacteriaceae* group and it is usually abbreviated as *E group.* It is an opportunistic bacterium and the most frequently isolated organism in patients with bacteremia and urinary infections. In some cases, *E. coli* infections result in death (Owrangi *et al*., 2018).

The ability of UPEC to ascend from the bladder to the upper urinary tract likely involves many genetic and environmental factors, one of which is thought to be the up-regulation of P pili and the corresponding down-regulation of type 1 pili. P pili, which are encoded by the pap operon, mediate the adhesion of UPEC to kidney tissue, resulting in pyelonephritis. This binding is dependent on the expression of the pilus tip adhesin, PapG. Moreover, ExPEC strains may have several virulence factors, such as host defences subverting mechanisms, iron acquisition systems, toxins, and adhesion, all of which may be important factors in host infection.

Furthermore, bacteria without virulence factor-coding genes in the bacterial chromosome may acquire them by transmission from bacteria with virulence factor-coding genes, as a result of these genes usually being located on mobile genetic elements, such as pathogenicity islands or plasmids (Dale and Woodword, 2015). Several virulence factors may facilitate bacterial cells to infect and colonize the host and have been associated with bloodstream infections (Mora-Rillo *et al.*, 2015).

There are many antibiotics such as Trimethoprim-sulfamethoxazole, Ceftriaxone, Ciprofloxacin, Ampicillin, Aztreonam, Imipenem, or others that are available for the treatment of *E. coli* infections, but due to the carriage of *P. pilli* type C otherwise called papC and haemolysin A (HlyA) the effectiveness of many of these drugs are inhibited therefore the study sought to amplify PapC and hlyA virulence genes in *Escherichia coli* from clinical isolates in some major hospitals in Bayelsa State.

***Corresponding Author:** Tolulope Olukemi Alade
Department of Medical Laboratory Sciences, Faculty of Basic Medical Sciences, College of Health Sciences

EXPERIMENTAL SECTION/MATERIALS AND METHODS

This study was carried out at the Federal Medical Center Yenagoa and the Niger Delta University Teaching Hospital Okolobiri, both in the Yenagoa Local Government Area of Bayelsa State. Bayelsa State is located in the Niger Delta region of the South-South geopolitical zone of Nigeria.

The state is geographically located within latitudes 04° 15' north, 05° 23' South and longitudes 05° 22' west and 06° 45' east. It is situated within Delta State on the north, Rivers State on the east, and the Atlantic Ocean on the west and south. Yenagoa is the capital of Bayelsa State.

Ethical Clearance

Ethical clearance was obtained from the ethical committees of the Federal Medical Center Yenagoa and the Niger Delta University Teaching Hospital, Yenagoa, Bayelsa State.

Sample Collection

A total of one hundred and forty-five (145) clinical specimens were randomly selected, ranging from urine, endocervical swabs, high vaginal swabs, wound swabs, and sputum. These specimens were collected and immediately transported to the Niger Delta University Microbiology Laboratory for processing**.**

Isolation of *Escherichia coli*

The specimens collected were cultured onto Cystine Lactose Electrolyte Deficient (CLED) agar and incubated at 37^0 C for 24 hours. After 24 hours of incubation, the cultivated colonies were observed macroscopically and their colonial morphology was noted. *Escherichia coli* fermented lactose and appeared yellow on CLED.

Identification

Gram Staining

Colonies suspected to be *E. coli* were picked with a sterile wire loop and emulsified with normal saline in a clean glass slide. Allowed to air dry and heat-fixed by passing the slide over a Bunsen burner three times, then covered with the primary stain (crystal violet) for 1 minute and washed off with clean water. Then covered with Lugol's iodine for 1 minute and washed off with clean water; decolourized with acetone and washed off immediately with clean water. The slide was then covered with the counterstain (safranin) for 2 minutes and washed off with clean water. The slide was allowed to dry and examined microscopically using an X-100 oil immersion lens.

Citrate Utilization Test

The suspected colonies were inoculated into Simmon's citrate agar and incubated at 37 C for 48 hours. Citrate-negative organisms showed no change in the colour of the medium and remained green due to their inability to use citrate as their only source of carbon.

Indole Test

These suspected colonies were tested for their ability to breakdown the amino acid tryptophan by subculturing a colony into peptone broth and incubating overnight at 37 C with Kovac's reagent, which contains 4, para-dimethylaminobenzaldehyde as the active ingredient. Indole-positive organisms showed a pink ring at the surface of the broth.

Oxidase Test

The oxidase test was carried out by smearing a colony of the suspected organism on Whatman No. 1 filter paper soaked with an oxidase reagent. Oxidase-negative organisms showed no blue-purple colour within 10 seconds.

Kligler Iron Agar

This test was performed by inoculating the test organism from peptone broth into KIA by streaking the slope and stabbing the butt, and it was incubated overnight at 37°C using already prepared KIA.

Antibiotic Susceptibility Testing

The disk diffusion method of Kirby Bauer was used to test for the susceptibility of 20 *Escherichia coli* isolates (Bauer *et al.*, 1996) against the following 10 antimicrobial agents: Tarivid (10µg), Reflacine (10µg), Ciprofloxacin (10µg), Augmentin (30µg), Gentamycin (10µg), Streptomycin (30µg), Ceporex (10µg), Nalidixic acid (10µg), Septrin (30µg), and Ampicillin (30µg). Peptone broth containing the organisms was poured on the Nutrient agar plate and then the antibiotic disc was placed on the plate surface and incubated at 37 C for 24 hours. The resistance showed no clear zones and the inhibition zones were recorded.

Molecular Analysis

DNA Extraction (boiling method)

Overnight culture of the pure isolates in peptone broth was transferred into a 2 ml Eppendorf tube and spun at 14000 rpm for 3 minutes in a microcentrifuge. The supernatant (peptone) was discarded, and 1000 l of 0.5% physiological saline was added to the sediment and mixed on an Etech XH-B Vortex machine. The supernatant (physiological saline) was discarded, and the process was repeated. After which, 1000ul of DNA elution buffer was then added to the sediment and mixed on the Eltech XH-B Vortex machine, and then heat shocked for 20 minutes at 95^oCand immediately fast cooled in the freezer for 10 minutes to lyse the cytoplasm to release the DNA. The Eppendorf tubes were then removed from the freezer and then centrifuged for 3 minutes at 14000 rpm. This centrifugation caused other macromolecules of the

isolates, such as protein, carbohydrate, and lipid, to precipitate as sediment at the bottom of the Eppendorf tubes, while the DNA came up as the supernatant. 500 l of the supernatant was then carefully aspirated into 1.5 ml Eppendorf tubes and stored at -2 oC until it was time for PCR analysis.

DNA Quantification

The extracted bacterial DNA was quantified using a Nanodrop 100 model with its corresponding software installed on a computer system. 2ul of the extracted bacterial DNA was used. The purity and quantity of the DNA extract were read off of the equipment.

AMPLIFICATION OF PapC and HlyA

The PCR amplification of the papC (200 bp) and HlyA (1,177 bp) genes was performed in a 40-l reaction mixture (Taq polymerase, dNTPs, MgCl, buffer), with forward and reverse primers at a concentration of 0.4 nm in volume supplied by Inqaba Biotech, South Africa. Water and the extracted bacterial DNA as templates. The genes were amplified using the set conditions as follows: 94^0C for 30 seconds, 540C for 30 seconds, 72^0C for 5 minutes, for 35 cycles on the Gene Amp PCR System 9700 Applied Biosystems Thermal Cycler. PapC was amplified at the annealing temperature of 61 ^{o}C, while HlyA was at 54^{o}C.

Agarose Gel Electrophoresis

The PCR products were resolved on gel electrophoresis using 1.5% agarose that was tinted with ethidium bromide and visualized on an ultraviolet trans-illuminator. The sizes of the DNA were determined using a Mass ruler high-range molecular DNA ladder (See appendix).

Statistical Analysis

Data were analyzed using Chi-Square with GraphPad Prism software to compare the number of bacteria isolated from males and females concerning the sources of these bacteria. A P value of 0.0001 was observed and considered to be statistically significant.

RESULTS

Table 1: Distribution of bacterial isolates by specimen

Bacterial Isolates	High Vagina Swab (%)	Wound swab (%)	Urine (%)	Sputum (%)	Endocervical swab (%)	Total (%)
E.coli	5(25.0)	-	12(60.0)	-	3(15.0)	20(13.8)
Proteus mirabilis	-	1(9.1)	9(81.8)	1(9.0)	-	11(7.6)
Klebsiella species	20(51.3)	-	10(25.6.5)	9(23.0)	-	39(26.9)
S. aureus	6(17.6)	17(50.0)	11(32.4)	-	-	34(23.4)
Pseudomonas species	-	18(43.9)	11(26.8)	12(29.3)	-	41(28.3)
Total%	**22(15.2)**	**31(18.1)**	**67(51.1)**	**22(15.2)**	**3(2.1)**	**145(100)**

Table 2: Distribution of isolates obtained by gender

SPECIMEN	MALE (%)	FEMALE (%)	TOTAL (%)
Urine	**20(29.9)**	**47(70.1)**	**67(46.2)**
High Vagina swab	**-**	**22(100)**	**22(15.2)**
Wound swab	**20(64.5)**	**11(35.5)**	**31(31.4)**
Endocervical swab	**-**	**3(100)**	**3(2.1)**
Sputum	**9(40.9)**	**13(59.1)**	**22(15.2)**
Total	**49(33.8)**	**96(66.2)**	**145(%)**

Table 3: Biochemical reaction of isolates from Clinical samples

ISOLATE NAMES	GRAMS STAIN	CITRATE	INDOLE	OXIDASE	CATALASE	COAGULAS	GLUCOSE	LACTOSE	SLOPE	BUTT	H_2S	GAS	MOTILITY
E.coli	_	_	+	_	NA	NA	+	+	Y	Y	-	+	+
Pseudomonas Aeruginosa	_	+	_	+	NA	NA	-	-	R	R	-	+	-
Proteus Mirabilis	_	+	_	_	NA	NA	+	+	R	Y	+	+	+
Staphylococcus Aereus	+	NA	NA	NA	+	+	NA	-	NA	NA	NA	NA	NA
Klebsiella pneumonia	_	+	_	_	NA	NA	+	+	Y	Y	-	+	-

Key:
- : Negative
+ . Positive
NA: Not Applicable
Y: Yellow
R: Red

Table 4: Showing the pattern of susceptibility and resistance of *Escherichia coli* in percentage distribution

CODE	NA **R (%) 18(90)**	PEF **R (%) 12(60)**	CN **R (%) 9(45)**	AU **R (%) 14(70)**	CPX **R (%) 11(55)**	STX **R (%) 16(80)**	S **R (%) 4(20)**	PN **R (%) 14(70)**	CEP **R (%) 11(55)**	OFX **R (%) 8(40)**
O1	R	R	R	R	R	R	S	R	R	S
O2	R	R	R	R	R	R	S	R	S	S
O3	R	R	S	S	S	S	S	R	R	S
O4	R	R	R	R	R	R	R	S	R	S
O5	S	R	R	R	R	R	S	S	R	R
O6	R	R	R	R	R	R	S	R	S	R
O7	S	R	R	R	S	R	S	R	S	R
O8	R	R	R	R	R	R	S	R	R	S
O9	R	R	S	R	S	R	S	R	S	S
O10	R	R	S	R	S	R	S	R	R	S
O11	R	R	S	S	R	R	S	R	S	S
O12	R	S	S	S	S	S	S	S	S	S
O13	R	S	S	S	S	R	S	S	S	S
O14	R	S	S	S	S	S	S	S	S	R
O15	R	S	S	S	S	S	S	S	S	S
O16	R	S	S	R	R	R	R	R	R	R
O17	R	R	R	R	R	R	S	R	R	R
O18	R	S	R	R	S	R	R	R	R	S
O19	R	S	S	R	R	R	S	R	R	R
O20	R	S	S	R	R	R	R	R	R	R

Key: **OFX:** Tarivid, **PEF:** Reflacine, **CPX:** Ciprofloxacin, **AU:** Augmentin, **CN:** Gentamicin**, S:** Streptomycin, **CEP:** Ceporex, **NA:** Nalixidic acid, **SXT:** Septrin, **PN:** Ampicillin. **R:** Resistant**, S:** Susceptible.

Table 5: Distribution of Bacterial Isolates by age and gender

MALE (%)						**FEMALE (%)**				
Age Ranges (yrs)	***E.coli* (%)**	***Klebsiella spp* (%)**	***Proteus spp*(%)**	***Sauries* (%)**	***Pseudomonas Aeruginosa* (%)**	***E.coli* (%)**	***Klebsiella spp.* (%)**	**Proteus spp. (%)**	***S.aureus* (%)**	***Pseudomonas aeruginosa*(%)**
0-10	0 (%)	0%	0%	0	0	0	0	0	0	0
11-20	2(50%)	0	1(25)	0	1(25)	0	2(16.7)	1(8.1)	4(33)	5(41.70)
21-30	1 (14.3)	2(28.6)	0	2(28.6)	2(28.6)	2(10)	10(50)	0	5(25)	3(15)
31-40	0	0	0	4(100)	0	3(15.7)	5(26)	1(5.3)	8(42)	2(10.5)
41-50	1(16.7)	0	2(33.3)	0	3(50)	3(15.8)	6(31.6)	2(10.5)	4(21.1)	4(21.1)
51-60	2 (22)	3(33)	0	0	4(44)	0	7(41.2)	1(5.9)	6(35.3)	3(17.6)
61-70	1(9.1)	2(18.2)	1(9.1)	2(18.2)	5(45.5)	3(13.6)	8(36.4)	0	7(31.8)	4((18.2)
71-80	1(8)	2(16.7)	2(16.7)	2(16.7)	5(41.17)	1(11.1)	0	0	8(88.9)	0
81-90	0	0	0	0	0	0	0	0	0	0
Total	8	9	6	10	20	12	38	5	42	21

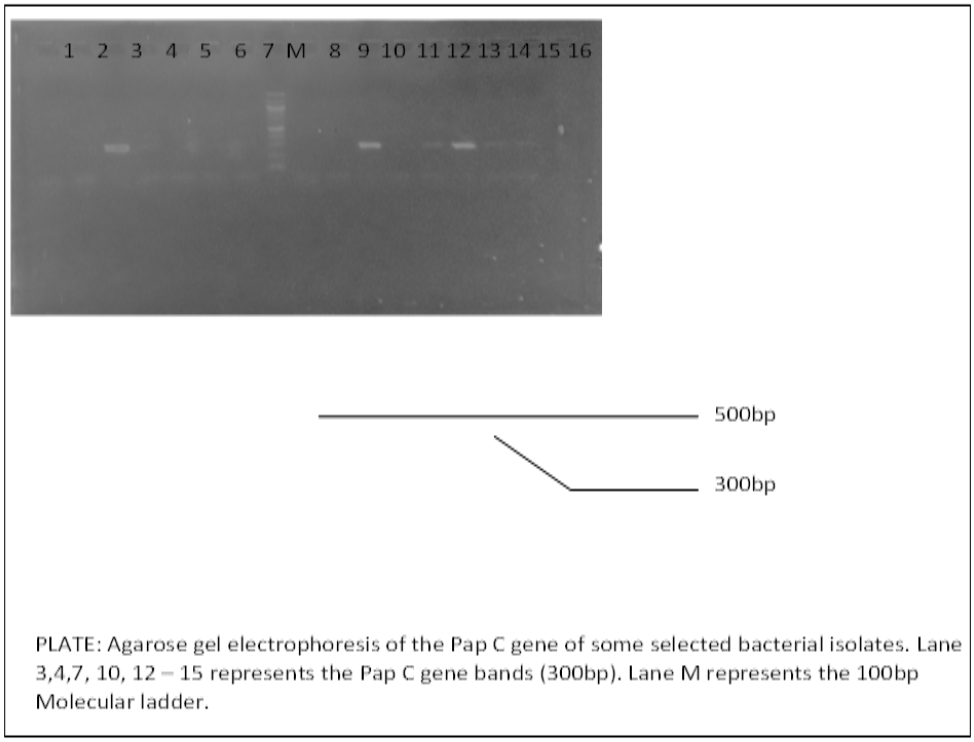

PLATE: Agarose gel electrophoresis of the Pap C gene of some selected bacterial isolates. Lane 3,4,7, 10, 12 – 15 represents the Pap C gene bands (300bp). Lane M represents the 100bp Molecular ladder.

Plate 1

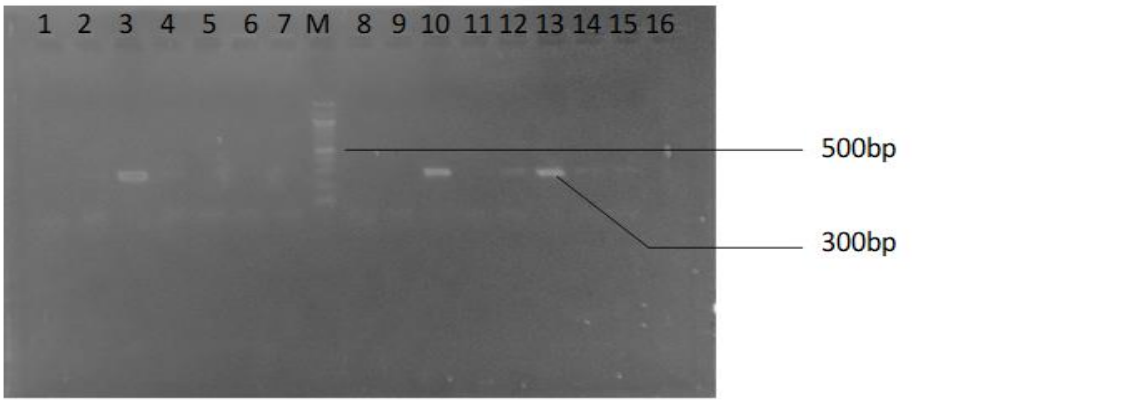

PLATE: Agarose gel electrophoresis of the Pap C gene of some selected bacterial isolates. Lane 3,4,7, 10, 12 – 15 represents the Pap C gene bands (300bp). Lane M represents the 100bp Molecular ladder.

Plate 2

DISCUSSION

The study showed that the bacterial isolates gotten from the various clinical specimens were *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis,* as shown in the isolation and identification of the studied organism.

Based on the site of infection from several clinical specimens, including urine (12%), endocervical swabs (15%), and high vaginal swabs (25%) the dispersion of the *Escherichia coli* was determined. Based on the gender, there was greater bacterial distribution among the female patients compared to their male counterparts. The statements are in agreement with the work of Dielubanza and Schaeffer (2011) which showed that *E. coli* was the most common urinary pathogen, causing 60–90% of urinary infections. Again, the high incidence of infection recorded among females might be attributed to the fact that women are mostly asymptomatic, which translates to a higher degree of functional immunity in comparison to their male counterparts.

In this study, a high proportion of the isolates were also resistant to Nalidixic acid (90%), Reflacine (60%), Augmentin (70%) and Septrin (80%), Ampicillin (20%) and Ceporex (70%) while Gentamicin, Streptomycin, and Tarivid showed low levels of resistance (45%), (80%), and (55%) respectively. This high proportion of resistance levels exhibited could be due to the fact that *E. coli* is naturally resistant to beta-lactam ring-containing drugs, including broad-spectrum Cephalosporins, Quinolones, Chloramphenicol and Tetracycline, mainly because of their low cell wall permeability.

However, from this study, the high resistance to Nalidixic acid was (90%) and was contrary to the work done by Lim *et al.,* (2009), who reported that out of 47 *Escherichia coli* they isolated from various public hospitals in Malaysia, they observed the resistance to Nalixidic acid to be (28%). Nevertheless, the result of this study was in conformity with the research carried out by Alhaji *et al*., (2007) that reported that resistance to Nalidixic acid was (68.6%).

The lowest resistance exhibited by *E.coli* in this study was to Ampicillin (20%) and this was contradictory to the report given by Adzitey *et al*., (2013). Differences in the percentage of antibiotic resistance observed by different authors may be due to differences in production systems, area of study, type and number of samples analyzed.

Out of the fifteen PCR products of *E. coli* that were resolved on agarose in this study, only eight (53.3%) confirmed the presence of the papC gene at 300 bp, as shown on Plate 1, and seven (87.5%) of these eight papC-carrying *Escherichia coli* isolates were susceptible to Ciprofloxacin.

PapC and HlyA have been studied to be virulence genes that can either aid resistance or lower the resistance capacity of *Escherichia coli* to some groups of antibiotics, such as Ciprofloxacin and fluoroquinolones. According to Karami *et al.* (2008), it was reported that the lower the prevalence of these genes in *Escherichia coli*, the more resistant *E. coli* became to the mentioned antibiotics.

Karami *et al.,* (2017) showed in the work they carried out in 2017 that antibiotic resistance was linked to the carriage of papC virulence genes in commensal and uropathogenic *Escherichia coli* from infants and young children, and so far, this study has been in conformity with theirs as many of the papC-carrying *E. coli* were resistant to other drugs except for Ciprofloxacin.

Numerous investigations on *Escherichia coli*, including those by Karami *et al.,* (2016), Harwalker *et al.,* (2014), and many more conducted earlier, found that *E. coli* developed its pathogenicity through adhesion, the secretion of toxins, and other mechanisms, such as the two genes: The study's examination of the pyelonephritis-associated pili (pap) C and -hemolysin (hlyA) represented adhesion and toxin secretion as mechanisms of establishing pathogenicity, respectively.

In conclusion, the two major health institutions in Bayelsa were the sources of clinical isolates for the study of two *Escherichia coli* virulence genes, papC and hlyA and their contributions to resistance and susceptibility. It was discovered that clinical isolates had a significant frequency of multi-drug resistance.

REFERENCES

- Adzitey, F., Rusul, G., & Huda, N. (2013). Prevalence and antibiotic resistance of *Salmonella* serovars in ducks rearing and processing environments in Penang, Malaysia. *Food research international*, 45(45), 947-952.
- Alhaji, N., Mariana, S., Raha, A., & Ishak, Z. (2007). Prevalence of antibiotics resistance among *Escherichia coli* from different sources in Malaysia. *International Journal of poultry Science*, 6(6), 293-297.
- Dale, A. P., & Woodford, N. (2015): Extra-intestinal pathogenic *Escherichia coli* (ExPEC): Disease, carriage and clones. *Journal of Infectious Disease*, 71, 615–626.
- Dharmalingam, S., Rao, A., Jayaraman, G., & Thyagarajan, S. (2003). Relationship of plasmid profile with antibiotic sensitivity pattern of *Helicobater pylori* isolates from peptic ulcer patients in Chennai. *Indian Journal of Medical Microbiology, 21*(3), 251-261.
- Dielubanza, E., & Schaeffer, A. (2011). Urinary tract infections in women. *Medical and Clinical Journal of North America*, 95, 37-41.
- Harwalkar, A., Gupta, S., Rao, A., & Srinivasa, H. (2014). Lower prevalence of hlyD, papC andcnf-1 genes in ciprofloxacin-resistant Uropathogenic *Escherichia coli* than their susceptible counterparts isolated from Southern India. *Journal of Infection and Public Health*, 7, 413-419.
- Harwalkar, A., Gupta, S., Rao, A., & Srinivasa, H. (2014). Lower prevalence of hlyD, papC andcnf-1 genes in ciprofloxacin-resistant uropathogenic *Escherichia coli* than their susceptible counterparts isolated from Southern India. *Journal of Infection and Public Health*, 7, 413-419.
- Karami, A., Romano, N., Galloway, T., & Hamzah, H. (2016). Virgin microplastics cause toxicity and modulate the impacts of phenanthrene on biomarker responses in Africa cat fish (*Clarias gariepinus*). *Environmental Research*, 151, 58-70.
- Karami, N., Hannoun, C., Adlerberth, I., & Wold, E. (2008). Colonization dynamics of ampicillin-resistant *Escherichia coli* in the infantile colonic

microbiota. *Journal of Antimicrobial Chemotherapy*, 62(4), 703–708

- Karami, N., Wold, A. E., & Adlerberth, I. (2017). Antibiotic resistance is linked to carriage of papC and iutA virulence genes and phylogenetic group D background in commensal and uropathogenic Escherichia coli from infants and young children. *European Journal of Clinical Microbiology & Infectious Diseases*, *36*(4), 721-729.
- Karlosky, A., Zhanel, G., Harding, K., Carrie, A., Mazzulli, T., & Low, D. (2000). *Antimicrobial Agents Chemotherapy*, 44(3), 1089-1092.
- Mora-Rillo, M., Fernández-Romero, N., Navarro-San Francisco, C., Díez-Sebastián, J., Romero-Gómez, M. P., Arnalich Fernández, F., Arribas López, J. R., & Mingorance, J. (2015). Impact of virulence genes on sepsis severity and survival in *Escherichia coli* bacteremia. *Virulence*, 6, 93–100.
- Owrangi, B., Masters, N., Kuballa, A., O'Dea, C., Vollmerhausen, T. L., & Katouli, M. (2018). Invasion and translocation of uropathogenic *Escherichia coli* isolated from urosepsis and patients with community-acquired urinary tract infection. *European Journal of Clinical Microbiology*, 37, 833–839.

Cite This Article: Tolulope Olukemi Alade, Chindinma Daokoru-Olukole, Oluwasegun Matthew Abidogun (2023). Molecular Detection of PapC and hlyA Virulence Genes in Escherichia coli from Clinical Isolates in Bayelsa State, Nigeria. *East African Scholars J Med Surg, 6*(4), 134-140