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Pathogenic potential of *Escherichia coli* from various sources in Port Harcourt, Rivers State, Nigeria

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ABSTRACT: Despite its occurrence as a commensal, *Escherichia coli* is also notorious as a pathogen. One variation between these commensals and pathogens is the presence of specific factors one of which are the pathogenicity islands. One of the most commonly occurring of these is the PAI IV_{536}. Potentially pathogenic species have been described in non-clinical settings. This often raises concerns on the role these environments play in transmission. This study therefore aimed at comparing the pathogenic ability of *E. coli* isolates from clinical and non-clinical sources based on the presence of the PAI IV_{536} marker. Thirty-five *E. coli* isolates were analyzed in this study. Following DNA extraction by boiling, the PAI IV_{536} gene fragment was amplified following standard procedure using the F5′-AAGGATTCGCTGTTACCGGAC-3′ and R5′-TCGTCGGGCAGCGTTTCTTCT-3′ primer pair. Of the 35 isolates, 13 were from clinical sources and 22 from non-clinical sources. In total, 25.7% (9/35) of the *E. coli* isolates in this study were found to possess the PAI IV_{536} gene. Clinical isolates had a much higher association of 61.5% with the PAI IV_{536} gene than non-clinical which only had a 4.5% representation of the PAI IV_{536} gene. This study reports on the detection of PAI IV_{536} in *E. coli* isolates from Port Harcourt, Rivers State Nigeria and a lower association of this pathogenicity marker with non-clinical isolates.

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Escherichia coli is widely known as a commensal found in association with various human and non-human systems particularly the gastrointestinal tract, where it exists without causing any observable harm (Allocati *et al.,* 2013, Fratamico *et al.,* 2016). These organisms exist as part of the normal flora of the intestines of humans but are routinely encountered in the environment due to faecal and wastewater discharges (Jang *et al.,* 2017). *E coli* is however also notorious as a pathogen, notable as the most common human Gram-negative bacterial pathogen isolated in the clinical microbiology laboratory (Poolman and Wacker 2016, Vila *et al.,* 2016). It is notorious for mediating a wide variety of infectious diseases ranging from mild self-limiting to more serious life-threatening conditions. It is a leading cause of urinary tract infections, diarrheal diseases, bacteremia and meningitis (Vila *et al.,* 2016, Poolman and Wacker 2016).Commensal and pathogenic *E. coli* differ in the presence of specialized pathogenic and virulence factors in the pathogenic strains. These factors are of a variety of types and include invasins, adhesins, toxins, iron-acquisition systems and type three secretion system (Mainil 2013). These pathogenic strains of *E. coli* commonly develop due to gene acquisition, which provides the acquiring organism with new traits and often a fitness advantage. The genome sizes of the pathogenic variants have been found to differ by up to a million base pairs (Croxen *et al.,* 2013), with an up to 20% variation found to occur between commensal and pathogen genomes (Ochman and Jones 2000). These extra genes which confer the pathogenic potential belong to a subset of genes referred to as the flexible gene pool. Gain or loss of such genes occurs at specific hotspots distributed all over the genome (Touchon *et al.,* 2009). Some of these virulence genes may be encoded in chromosomal pathogenicity islands (Chekabab *et al.,* 2013), which are a unique class of genomic islands. And these genomic islands have been found to be significant in defining *E. coli* (van Elsas *et al.,* 2011). Two studies have noted that a quarter of the genomic content of particular pathogenic strains was made up of genomic islands (Chaudhuri *et al.,* 2010, Perna *et al.,* 2001). Pathogenicity islands (PAIs) are distinct chromosomal mobile genetic elements, obtained by horizontal gene transfer which contribute to genome evolution (Oelschalaeger and Hacker 2004). These elements first described in *E. coli* (Schmidt and Hensel 2004) are known to carry virulence genes and, in some cases, antibiotic resistance genes. They are generally present in pathogenic strains but absent in non-pathogenic strains

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(Gal-Mor and Finlay 2006). PAIs are generally associated with specific sites of the chromosome and mobility genes and elements. Characteristically, they have a G+C content which varies from the surrounding core genome. Several PAIs exist with up to 8 described (Calhau *et al.,* 2015). One of such PAIs is the PAI IV_{536} initially associated with uropathogenic *E. coli* strain 536. PAI IV_{536} is a high pathogenicity island notable for its association with yersiniabactin, a siderophore system (Karczmarczyk *et al.,* 2011). This particular pathogenicity island has been found associated with intestinal pathogens, uropathogens, extraintestinal pathogens and even commensals and is often the most commonly detected PAI in enterobacteria (Karczmarczyk *et al.,* 2011, Dobrindt *et al.,* 2002, Sabate *et al.,* 2006, Koga *et al.,* 2014).

Quite often, potentially pathogenic species have been found in non-clinical settings such as poultry environments, ready-to-eat foods, drinking water systems and inanimate surfaces (Otokunefor *et al.,* 2018, Cookey and Otokunefor 2016, Agbagwa and Onyebule 2018). Often, they are found in association with high decrees of drug resistance. And concern has been expressed regarding the transfer of these isolates into the clinical settings and the potential human impact. Few studies have however set out to ascertain the actual pathogenic ability of isolates obtained. This study was therefore aimed at exploring the pathogenic ability of a panel of *E. coli* isolates based on the presence of a PAI IV_{536} marker.

MATERIALS AND METHODS

Bacterial Isolates: Thirty-five *E. coli* isolates were analyzed in this study. These isolates were obtained from the culture collection of the Bacteriology group of the Medical Microbiology Unit of the University of Port Harcourt.

DNA extraction: The boiling method (Oliveira *et al.,* 2014) was used for DNA extraction in this study. Pure bacterial colonies were boiled in 100µL of molecular grade water for 5 min and impurities removed by centrifugation at 10,000g for 5min. Bacterial DNA contained in the supernatant was then used in the molecular studies.

Detection of PAI marker: The PAI IV_{536} gene fragment was detected using previously described primers (Koga *et al.,* 2014). Each amplification reaction mix comprised of 2µl master mix (5x), 0.3µl each of forward and reverse primer (10 pmol/µl), 2µl of DNA template and 5.4µl of H_2O in a final volume of 10µl. The amplification protocol involved an initial denaturation at 95°C for 5 mins followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 56°C for 30 secs and extension at 72°C for 2 min. it ended with a final extension of 72°C for 10 mins. Following amplification, PCR products were visualized on 1.5% agarose gels stained with ethidium bromide.

RESULTS AND DISCUSSION

Source of Isolates: Identities of the 35 *E. coli* isolates had previously been confirmed using both phenotypic and molecular methods (Otokunefor *et al.,* 2019). Of these 35 isolates, 13 had been obtained from clinical sources and 22 from non-clinical sources.

Detection of PAI IV_{536} gene: In total, 25.7% (9/35) of the *E. coli* isolates in this study were found to possess the PAI IV_{536} gene. The rate of carriage of this gene was however found to differ based on source of the isolates. Clinical isolates were found to exhibit a higher carriage rate than non-clinical isolates (Figure 1), with a 57% difference in level of carriage.

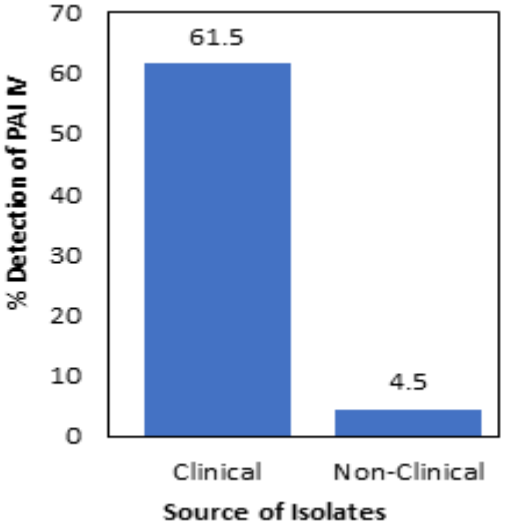

Fig 1: Presence of the pathogenicity island PAI IV_{536} gene marker in *E. coli* isolate

Eight pathogenicity islands have commonly been described in *E. coli* isolates. One of the more commonly occurring of these pathogenicity islands is PAI IV_{536}. Several studies exploring the prevalence of this island in various *E. coli* isolates have noted much higher rates (68% to 98.6%) than reported in this study (Sabate *et al.,* 2006, Najafi *et al.,* 2018, Samei *et al.,* 2016, Calhau *et al.,* 2015, Kryger *et al.,* 2015). In contrast, other studies reported lower rates similar to that obtained in this study ranging from 21% to 43% (Sabate *et al.,* 2006, da Silva *et al.,* 2017, Peerayeh *et al.,* 2018). This variation appears to be linked in some cases to the source of isolates. The studies reporting high rates predominantly explored the rate of PAI IV_{536} in uropathogenic *E. coli* (UPEC) isolates, while the lower rates were described in enteroinvasive *E. coli* (EIEC) and commensals. The low association

between PAI IV_{536} and the non-clinical isolates analyzed in this study is a welcomed finding as it could point at a lower ability of these isolates to cause disease in humans hence indicating a reduced risk from these isolates if transferred into clinical settings.

Conclusion: This study reports on the detection of PAI IV_{536} in *E. coli* isolates from Port Harcourt, Rivers State Nigeria. The lower association of this pathogenicity marker with non-clinical isolates is a welcomed finding. A larger scale study would be ideal to properly confirm the findings of this study.

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