

ASSESSMENT OF VIRULENCE POTENTIAL OF *ESCHERICHIA COLI* ISOLATED FROM CLINICAL AND NON-CLINICAL SOURCES IN PORT HARCOURT, NIGERIA.

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ABSTRACT

Escherichia coli commonly occurs as a normal flora found in a variety of gastrointestinal tracts. However, certain strains acquire virulence characteristics. Potentially pathogenic species have also been isolated in non-clinical environments. This often raises concerns on the role these environments play in transmission. This study aimed to assess the occurrence of virulence traits in *Escherichia coli* isolated from non-clinical and clinical sources in Port Harcourt, Nigeria. Two hundred samples from clinical (urine and stool) and non-clinical (soil and poultry) sources were analyzed. 8 isolation, purification and identification of *E. coli* isolates were performed using standard bacteriological and biochemical tests. Out of the 200 samples collected, 98 isolates (49%) were confirmed as *E. coli*. Confirmed *E. coli* isolates were then tested for virulence using standard phenotypic tests (protease, colicin, congo red, hemolysin, haemagglutination, adhesin and mannose resistant/sensitive haemagglutination). *E. coli* strains from clinical samples displayed a number of virulence traits ranging from biofilm producing capabilities (53%), congo red binding propensity (24%), colicin (34%), protease (15%) and hemolysin production (4%) compared to their non-clinical counterpart. All samples were negative for haemagglutination. The total virulence prevalence of *E. coli* was 81%. Clinical samples possessed more virulence traits (71%) than samples from non-clinical sources (29%), with urine having a higher occurrence among the clinical samples (36%). There is need for an in-depth study on virulence attributes of *E. coli* and ways to control these factors to minimize the rate of spread and the degree of infections.

Keywords: *Escherichia coli*, pathogenic, phenotypic tests.

INTRODUCTION

Escherichia coli is a very common and distinct organism with a very diverse phylogenetic makeup. It is largely a commensal inhabitant of the gastrointestinal tracts of several animals and healthy humans (Rojas-Lopez et al. 2018). However, it has been implicated in a wide range of infections spanning from intestinal, to extra-intestinal diseases (ExPEC) (Um et al., 2018). *E. coli* is a major cause of urinary tract

infections, diarrheal infections, bacteremia and meningitis (Jayan et al., 2020). It is responsible for about 90% community acquired and 50% hospital acquired UTIs (Wireko et al., 2017). It is the third most detected bacterial infection and a serious health issue after respiratory and gastrointestinal tract infections (Nandihal, 2015).

It has been proposed that pathogenic *E. coli* strains emanated from commensal ones by the uptake of virulence genes carried either chromosomally or extra-chromosomally (Sobhy et al., 2020). Commensal and pathogenic *E. coli* differ in terms of the virulence traits harboured in the pathogenic strains. These factors comprise but are not restricted to; invasins, adhesins, toxins, iron-acquisition systems and type three secretion system (Otokunefor and Nyema, 2019; Desvaux et al., 2020). These pathogenic *E. coli* emanate from gene acquisition, which confers the acquiring organism with novel features and more often, a fitness advantage. Although clinical outcomes may differ in fatality, pathogenic *E. coli* still pose a huge public health threat as they continue to evolve, gaining new traits, which often metamorphose in even more virulent strains (Gomes et al., 2021).

Potentially pathogenic species have however also been isolated from non-clinical environments such as poultry environments, ready-to-eat foods, drinking water systems and inanimate surfaces (Otokunefor et al., 2018). Whilst majority of the organisms encountered in these environments are thought to be innocuous, there is a possibility that such environments might serve as reservoirs of disease causing *Escherichia coli*. This study therefore set out to assess and compare the virulence potential of *Escherichia coli* strains from clinical and non-clinical sources in Port Harcourt, Nigeria.

MATERIALS AND METHODS

Ethical Consideration

Ethical approval was obtained from the ethical committee of the University of Port Harcourt Teaching Hospital (UPTH) where the clinical samples were obtained (UPTH/ADM/90/S.11/VOL.XI/1110).

Sampling and Processing

Two hundred (200) samples from clinical patients (urine and stool) and non-clinical sources (soil and poultry faeces) were

analyzed. All samples were cultured and isolates identified using the conventional microbiological procedures. In brief, samples showing characteristic *E. coli* colonies on Eosin methylene blue agar (EMB) (Himedia, India) were purified and their identities confirmed biochemically using standard biochemical tests.

Phenotypic Identification of Virulence Properties of *E. coli* Isolates

E. coli isolates were then tested to ascertain the presence of seven virulence traits using standard phenotypic tests. The phenotypic tests included protease test, colicin test, curli fimbriae test (Congo red), hemolysin test, haemagglutination test, adhesin (biofilm) test and mannose resistant/sensitive haemagglutination test.

Hemolysis Test

Hemolysis was determined by inoculating pure colonies of *E. coli* on 5% sheep blood agar. After 24h of incubation at 37°C, a positive hemolysin result was identified by the appearance of clear zones around colonies.

Adhesin test

Adhesin test (biofilm formation) was determined by growing the test isolates overnight in a liquid broth at 37°C. Two (2) µl of this broth culture was then inoculated on to a sterile microscopic slide placed in a sterilized Petri dish. The slides were then incubated at 28°C for 3h after which, each slide was held using a forcep and rinsed thrice with distilled water, dried at 28°C, stained with crystal violet and viewed under a light microscope. (Abd El-Baky, 2019). Regarding the biofilm forming ability of isolates, this capacity was further categorized as either strong, moderate, weak or non-adherent.

Protease test

Protease test involved the inoculation of a pure culture of the test isolate on skimmed milk agar. Positive results were identified by observable zones of clearance around colonies after a 24h incubation at 37°C.

Mannose resistant and mannose sensitive haemagglutination test

For mannose resistant and mannose sensitive haemagglutination test, a drop of group “O” blood was mixed with a drop of the test isolate on a slide and gently rocked for 5 minutes at 37°C to enable an even mixture. The slides were thereafter observed for clump formation indicating a positive haemagglutination reaction. Mannose sensitive haemagglutination was inferred by the absence of haemagglutination upon addition of 2% w/v of d-mannose to the positive haemagglutination slide. The presence of haemagglutination indicated mannose resistance.

Congo Red binding test

Congo red binding test involved culturing the tested isolates on agar plates containing 0.1% tryptone, 0.05% yeast extract, 0.002% coomassie brilliant blue, 0.004% congo red and 1.5% agar. The formation of red colonies after a 24h incubation was considered positive for curli production while white colonies inferred curli negative.

Colicin production test

To detect colicin production, isolates were cultured on the all purpose nutrient agar for 24

h at 37°C. After incubation, the plates were opened and inverted on a beaker containing chloroform vapors for 2 h. After the 2h incubation, the plates were set aside to expel chloroform for 30 minutes after which, the isolates were inoculated side by side to the original cultures. The isolates were thereafter inspected with growth inhibition denoting a positive result (Reichhardt *et al.*, 2015).

Statistical Analysis

SPSS 17 statistical software (SPSS Inc., Chicago, IL) was used for the statistical analysis. Pearson chi-square (X²) test was employed for comparing the percentages obtained for genotypic characteristics and virulence genes. P-value was considered significant when less than 0.05.

RESULTS

Identification of *E. coli* from various clinical and non-clinical sources.

Of the 200 samples collected, ninety-eight non-duplicate isolates were found to be *E. coli* in general, which represent a 49% prevalence. A higher occurrence (62%) of *E. coli* was detected in clinical samples as opposed to non-clinical samples (Table 1), with the least occurrence (10%) observed specifically from soil samples (Figure 1).

Table 1: Source variation in detection of *Escherichia coli*

Source	<i>E. coli</i> (n= 98)	
	No sampled	No Positive (%)
Clinical	100	62(62.0)
Non-clinical	100	36(36.0)
Total	200	98 (49.0)

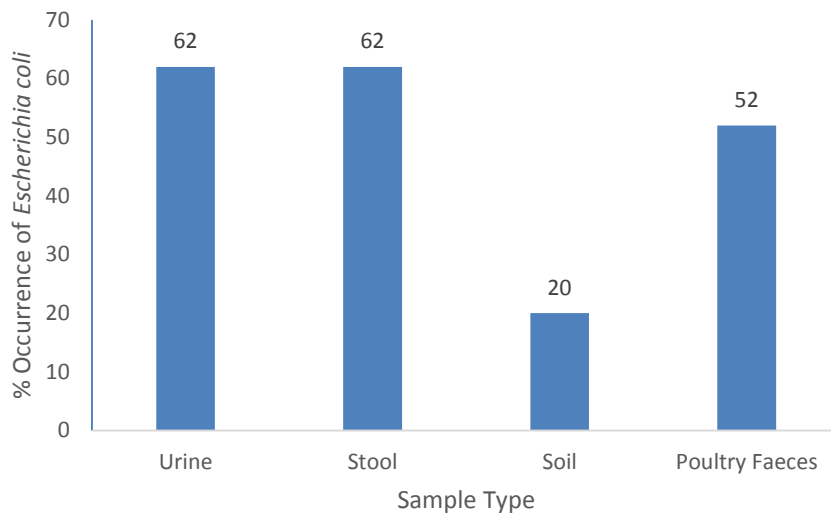


Figure 1: Distribution of *Escherichia coli* among study samples

Phenotypic screening for virulence markers on *E. coli* from clinical and non-clinical isolates.

An assessment of phenotypic traits revealed that 80.6% (79/98) of isolates harboured at least one of the phenotypic traits assayed for. The prevalence of the traits however varied, with zero occurrence of haemagglutination and mannose sensitivity in all isolates, while biofilm formation was the commonest trait (67.4%). Overall, clinical isolates had a higher occurrence of the virulence traits with colicin and hemolysin production found exclusively among the clinical isolates (Fig 2).

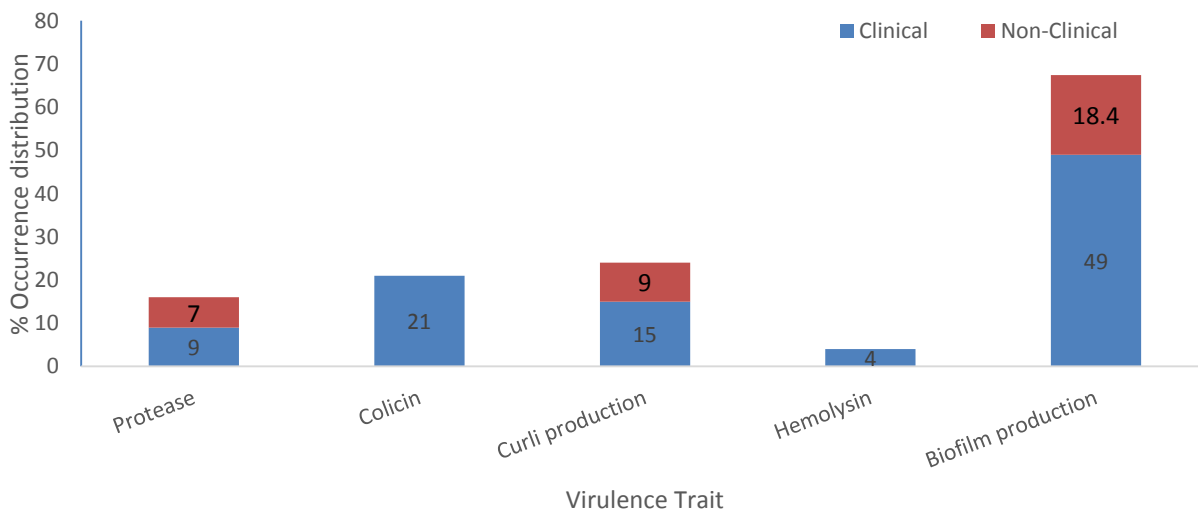


Figure 2: Comparison of virulence traits in clinical and non-clinical *E. coli*

An evaluation of the occurrence of phenotypic traits based on source of the sample showed that *E. coli* from poultry faecal matter which demonstrated curli production capability had the highest frequency of occurrence (26.9%) while for human stool, it was colicin production (25.8%). Generally, *Escherichia coli* from urine samples possessed more virulence traits (Table 2). Statistical analysis however showed no significant association between the virulence traits and the sample source.

Table 2: Overall distribution of the phenotypic virulence markers on *E. coli* isolated from various sources

Phenotypic tests for virulence properties	Urine (n=31)	Stool (n=31)	Soil (n=10)	Poultry faeces (n=26)	P value
Protease	3 (9.7%)	6 (19.4%)	5 (50.0%)	2 (7.7%)	0.012
Colicin	13 (41.9%)	8 (25.8%)	0 (0.0%)	0 (0.0%)	<0.001
Curli production	11 (35.5%)	4 (12.9%)	2 (20.0%)	7 (26.9%)	0.330
Hemolysin	3 (9.7%)	1 (3.2%)	0 (0.0%)	0 (0.0%)	0.254
Haemagglutination	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Mannose sensitivity	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	

Clinical isolates recorded higher frequency for strong/moderate biofilm production (50%). Samples from soil recorded the weakest biofilm producing capacity (60%) while poultry samples had the highest non-adherent strength (69.2%)(Table3). Statistical analysis showed a significant correlation between biofilm formingability and clinical source (P<0.001). Higher biofilm ability was noted in *E. coli* isolated from urine, with 17 isolates (54.8%) observed as strong/moderate biofilm producers.

Table 3: Overall adhesin occurrence of *E. coli* from clinical and non-clinical sources.

Biofilm Production	Urine (n=31)	Stool (n=31)	Soil (n=10)	Poultry faeces (n=26)	P value
None	6 (19.4%)	8(25.8%)	0 (0.0%)	18 (69.2%)	<0.001
Weak	8 (25.8%)	7 (22.6%)	6 (60.0%)	7 (27.0%)	
Moderate	8 (25.8%)	8 (25.8%)	3 (30.0%)	1 (3.8%)	
High	9 (29.0%)	8 (25.8%)	1 (10.0%)	0 (0.0%)	

DISCUSSION

The ability of a microorganism to cause diseases depends largely on their possession of one or more virulent factors. Bacteria tend to possess several virulent factors that aid their pathogenicity which are determined phenotypically by their hemolytic activity, mannose sensitivity, Congo red binding propensity, adhesin, colicin and protease production. In this study, *E. coli* present in human urine samples showed greater hemolysin production (3/31 9.7%) compared to *E. coli* from other sources. This agrees with the report by Abd El-Baky (Abd El-Baky et al., 2020), which stated that hemolytic activity was a common trait among *E. coli* associated with infections of the urinary tract. Hemolytic

activity is key in tissue damage and the interference with the local immune response. The result obtained from this study showed that *E. coli* from urine samples demonstrated greater Congo red binding propensity (11/31, 35.5%) than previous studies. Congo red binding assay is an epidemiological marker to distinguish between invasive and non-invasive *E. coli*, with the binding of dye linked with presence of virulence genes (Zahid et al., 2016; Reichhardt and Cegelski, 2018; Saha et al., 2020).

Protease production was common in faecal and soil isolates, but weak among urine isolates. Abd El-Baky et al., (2020) likewise reported greater protease production among *E. coli*

isolates from human stool samples. The production of this enzyme may be an indication of the capability of the isolates to cause damage to urinary and the intestinal cells, and this enzyme has been associated with the pathogenicity of *E. coli* (Abed et al., 2016). Tapadero et al., (2019) reported that certain proteases are pivotal in increasing the virulence of extraintestinal disease causing *E. coli*.

In this study *E. coli* isolated from urine samples showed greater colicin production (13/31, 41.9%) compared to *E. coli* isolated from faeces (8/31, 25.8%). Although, colicin production is an important character that has been observed in both clinical and non-clinical *E. coli* isolates (Cergole-Novella et al., 2015; Micenková et al., 2016; Abd El-Baky et al., 2020), the present study only observed colicin production among clinical isolates. There are three main ways colicins act against cells; either by causing breakdown of DNA or RNA, or the formation of pores which can lead to depolarization of cytoplasmic membranes and the inhibition of peptidoglycan synthesis (Hahn-Löbmann et al., 2019).

A high occurrence of strong biofilm forming ability was demonstrated by *E. coli* from the urine samples (9/31, 29.0%). Current results showed significant difference ($p < 0.005$) for adhesin production among the testisolates with relation to different sources, as it was noted that strong and moderate biofilm production was more prevalent among isolates from clinical samples (urine and stool). The prevalence of biofilm production was however low (27%) among the clinical isolates. Abd El-Baky et al., (2020) reported no significant difference ($p > 0.05$) for biofilm among the *E. coli* isolated clinical and non-clinical sources. Biofilm production is impacted by a number of factors including environmental parameters, constituents of the culture media and the manifestation of some biofilm promoting attributes such as curli and nonconjugative pili (Cergole-Novella, et al., 2015).

CONCLUSION

This study shows that most of the virulent organisms were predominant in clinical settings. The environment could have played a crucial role in the transfer of these organisms to non-clinical settings. Therefore, there is a need for a more elaborate study on molecular genes responsible for the virulence attributes of *E. coli* and ways to checkmate these factors to minimize the rate and the severity of infections.

Statements and Declarations

Competing Interests

All authors declare that they have no conflict of interests.

Ethical Statement

Ethical approval was obtained from the ethical committee of the University of Port Harcourt Teaching Hospital (UPTH) where the clinical samples were obtained (UPTH/ADM/90/S.11/VOL.XI/1110).

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