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Molecular detection of virulence and resistance markers in *Escherichia coli* and *Staphylococcus aureus* isolated from Nigerian currency

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Abstract

Potentially pathogenic bacteria are routinely linked with different currencies worldwide. Not much focus has been on the actual pathogenic potential of these bacteria. This is essential as the risk would depend on whether the bacteria associated with the currency is a commensal or pathogen. This study was therefore aimed at exploring the virulence potential of *Escherichia coli* and *Staphylococcus aureus* isolated from specific Nigerian currency denominations. Presumptive *E. coli* and *S. aureus* isolates were obtained from the samples and their identities confirmed genotypically. A total of seven virulence and resistance genes were tested for (*aggR, eae, ast, van, cat, pvl, icaA*). Three of these were *E. coli* virulence genes (*aggR, eae, ast*), two *S. aureus* virulence (*pvl, icaA*) and two *S. aureus* resistance (*van, cat*). Sixty presumptive *E. coli* and *S. aureus* (30 each) were isolated and purified. Of these, 9 (30%) were confirmed as *E. coli* following genotypic identification and 7 (23%) as *S. aureus*. An assessment of the virulence potential of *E. coli* showed 100% detection of the *ast* gene, 55.6% detection of *aggR* and 44.4% detection of *eae*. For *S. aureus*, a much lower frequency of test genes was found with rates of 41.7%, 16.7%, 16.7% and 8.3% for *pvl, icaA, van* and *cat* respectively. This study therefore reports a low frequency of *S. aureus* virulence genes. The *E. coli* strains however, rather than being innocuous carry virulence factors. Furthermore, some of these have a known association with mobile genetic elements and hence a capacity to transform harmless commensal strains to pathogens.

Keywords: *Virulence potential, E. coli, S. aureus, Naira, Nigeria*
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I. INTRODUCTION

The association of potentially pathogenic bacterial strains with various currencies worldwide has long been noted, with earliest reports from as far back as 1972 observed from the PubMed database (Abrams and Waterman, 1972). The focus of different studies has often been generally on detecting and identifying all non-fastidious bacteria present on various currencies worldwide (Orababa *et al.*, 2021). While this provides key epidemiological data, with respect to prevalence and distribution, it doesn't give much information with respect to virulence potential of organisms or the presence of antibiotic resistance determinants. And this information on virulence potential of key bacteria from environmental sources appears limited.

Several key factors are associated with bacterial virulence. These could differ from species to species but in general belongs to one of several groups which cover adhesins essential in attachment and colonisation, invasins necessary for entry, evasins needed for establishment of infection and toxins. A number of studies have actually gone further to assess virulence potential in bacteria isolated from various currency notes (Kumar *et al*., 2009, Thiruvengadam *et al*., 2014), but studies were more likely to screen for specific resistance genes rather than virulence genes (Jalali *et al*., 2015; Heshiki *et al*., 2017; Alfadil *et al*., 2018), even those using metagenomic approach. An assessment of the virulence potential of organisms is essential to ascertain if these strains of bacteria are pathogens or commensals. This is key as the risk associated with infection would differ depending on the type infecting strain. Such a study has not previously been carried out in our locale.

This study was therefore aimed at exploring the virulence potential of *Escherichia coli* and *Staphylococcus aureus* isolated from specific Naira denominations circulating in Rivers State, Nigeria.

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II. Materials And Methods

A. Isolation and identification of test strains

Presumptive *E. coli* and *S. aureus* isolates were obtained from specific Nigerian currency (Naira) denominations by culturing to selective media Eosin methylene blue (EMB) and Mannitol salt agar (MSA) respectively following sample processing as described by Vriesekoop *et al*., (2010). Characteristic colonies were then purified and stored.

Following this, DNA was obtained from pure cultures using the boiling method (Ajuga *et al.*, 2021). In brief, pure cultures were emulsified in 100 µl volume of sterile DNase free distilled water. The mixture was then boiled for 5 mins, followed by centrifugation at 10 000g for 5 mins to get rid of impurities contained in the sediment. The supernatant containing the DNA was then transferred to a fresh Eppendorf and used for further analysis.

Isolate identities were confirmed genotypically using *E. coli* specific 16s primers (F 5'-GACCTCGGTTTAGTTCACAGA-3' and R 5'-CACACGCTGACGCTGACCA-3') and the nuc primer (F 5'-GCGATTGATGGTGATACGGTT-3' and R 5'-AGCCAAGCCTTGAACGAACTAAAGC-3') for *E. coli* and *S. aureus* respectively. Similar amplification protocols were used for both primers and involved initial denaturation at 95°C for 5 mins, followed by 30 cycles of denaturation (95°C for 30 secs), annealing (55°C for 30 secs) and elongation (72°C for 1 min). Final elongation was done for 10 mins at 72°C.

Amplification products were then visualized following separation on a 1.5% agarose gel.

B. Detection of virulence and resistance profile of isolates

A total of seven virulence and resistance genes were tested for (Table 1). Three of these were *E. coli* virulence genes (*aggR*, *eae* and *ast*), two *S. aureus* virulence genes (*pvl* and *icaA*) and two, *S. aureus* resistance genes (*van* and *cat*).

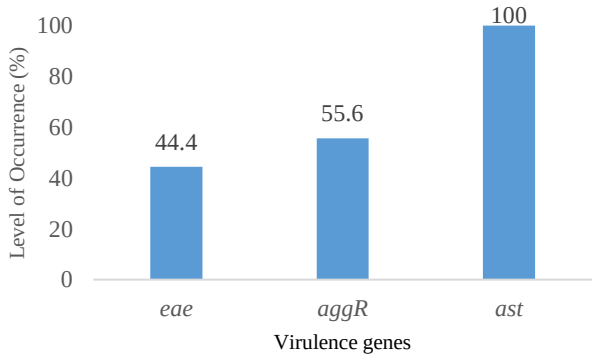

Figure 1. Variation in frequency of virulence genes in confirmed *Escherichia coli* isolated from specific Naira denominations

Table 1. Primers used to amplify specific virulence and resistance genes

Gene	Primer Sequence	Product Size	Ref
	Escherichia coli		
aggR	GTATACACAAAAGAAGGAAGC ACAGAATCGTCAGCATCAGC	254 bp	Bisi-Johnson *et al*., 2011
eae	ATGCTTAGTGCTGGTTTAGG GCCTTCATCATTTCGCTTTC	248 bp	Bisi-Johnson *et al*., 2011
ast	GCCATCAACACAGTATATCC GAGTGACGGCTTTGTAGTCC	106 bp	Bisi-Johnson *et al*., 2011
	Staphylococcus aureus		
van	GGGAAAACGACAA TTGC GTACAATGCGGCCGTTA	732 bp	Phukan *et al*., 2016
cat	AGTTGCTCAATGTACCTATAACC TTGTAATTCATTAAGCATTCTGCC	547 bp	Abdolmaleki *et al*., 2019
pvl	ATCATTAGGTAAAATGTCTG GACATGATCCA GCATCAAGTGTATTGGATAG CAAAAGC	433 bp	Karmakar *et al*., 2018
icaA	GACCTCGAAGTCAATAGAGGT CCCAGTATAACGTTGGATACC	814 bp	Bhowmik *et al*., 2021

aggR- transcriptional activator for EAEC aggregative adherence fimbria I expression; *eaeA*-*E. coli* attaching and effacing; *astA*-EAEC heat-stable enterotoxin; *icaA*- intracellular adhesion

III. Results

Sixty bacterial isolates were isolated and purified, thirty isolates for each type of *E. coli* and *S. aureus*, only 9 (30%) were positive for *E. coli* after genotype recognition and 7 (23%) for *S. aureus*. An assessment of the virulence potential associated with the confirmed *E. coli* isolates showed that the *ast* gene was detected in all isolates while *eae* gene was the least commonly occurring (Figure 1). Co-occurrence of these virulence genes were observed for majority of the isolates (6/9, 66.7%). Levels of co-occurrence was evenly distributed as 33.3%. of isolates exhibited co-occurrences of 2 and 3 of the virulence genes each. Co-occurrence of *aggR* and *ast* occurred in 2 cases as opposed to the 1 case of *eae* and *ast*.

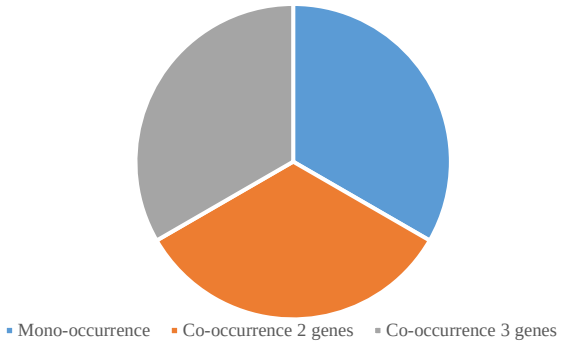

Figure 2. Distribution of co-occurrence of virulence genes in confirmed *E. coli* isolated from specific Naira denominations

For the *S. aureus*, a much lower occurrence of test genes was found with none of the virulence or resistance genes assayed for detected in all isolates (Figure 3) and levels of occurrence ranging from 8.3% to 41.7%. Co-occurrence of test genes occurred only in 1 isolate and involved a co-occurrence of the two antibiotic genes only.

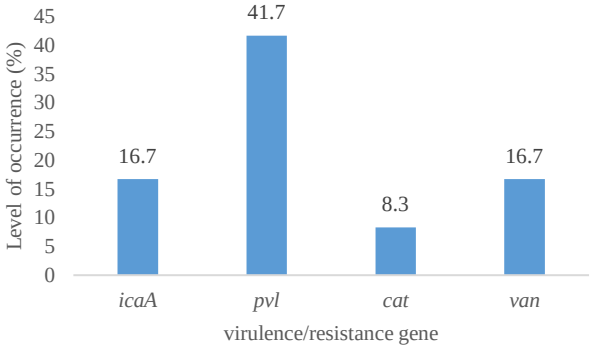

Figure 3. Variation in frequency of virulence and resistance genes in confirmed *Staphylococcus aureus* isolated from specific Naira denominations.

IV. Discussion

Though notorious as pathogens, both *Escherichia coli* and *Staphylococcus aureus* also occur as commensals with little risk to humans. For example, *E. coli* K12 a lab strain is considered a relatively innocuous risk group (RG) 1 organism and can be handled in a Bio-safety Lab1 (BSL1) facility such as an undergraduate microbiology laboratory while most other pathogenic *E. coli* are classed as RG2 organisms. When reporting on the association of bacteria with currency in order to determine possible roles of these currency as fomites in the transmission of disease-causing agents, it is essential to assess their disease-causing risk.

The presence of virulence factors is one of the main differences between commensal and pathogenic strains. These are varied and of different types. This study involved the detection of the *aggR*, *eae* and *ast* virulence determinants of *E. coli* which are responsible for control of expression of an Enteroaggregative *Escherichia coli* (EAEC) fimbriae, strain adherence and a heat stable toxin respectively. With pathogenic *E. coli*, specific virulence factors are usually found in association specific *E. coli* pathotypes (Kuhnert *et al.*, 2000, Pakbin *et al*., 2021). This current study reports a 100% representation of the *ast* gene. Its gene product the EAEC heat-stable toxin (EAST) was so named for its initial detection from EAEC strains but has since been associated with all enteric *E. coli* pathotypes (Veilleux and Dubreuil, 2006). Though previously reported to also be associated with commensals (Kaper *et al*., 2004), a more recent study (Ellis *et al.*, 2020) comparing virulence factors between lineages associated with an infection and carriage found a clear association of this toxin with the infection strains. This would indicate that all *E. coli* isolated in this study were pathogenic obtained possibly from faecal rather than environmental sources.

Detection of *eae* possibly indicates the presence of an EPEC or EHEC strain since the afimbrial adhesin intimin, *eae* encodes for is usually typical of these pathotypes (Meng *et al.*, 1998). The *eae* gene though is associated with the locus of enterocyte effacement (LEE) pathogenicity island and since the presence of one gene in a pathogenicity island is usually indicative of all other virulence determinants, this means that 44.4% of the *E. coli* in this study carry in addition, a type III secretion system and its effector proteins.

The *aggR* on the other hand encodes an activator for the aggregative adherence fimbriae I which is associated specifically with EAEC and is also regarded as a global virulence regulator (Dias *et al.*, 2020). This gene is significant as it is associated with the p*AA* plasmid (Cabal *et al.*, 2016). This association with a potentially mobile genetic element could point at an added risk of commensal strains becoming pathogenic. Additionally, whereas *ast* gene is also associated with commensals, the AggR regulon has been specifically associated with disease (Kaper *et al.*, 2004).

For *S. aureus*, a low association was noted with both virulence and resistance genes. The virulence gene which was detected in 41.7% of isolates, pvl, encodes the Panton-Valentine leucocidin. This is a cytotoxin that has potentially been associated with life-threatening skin and soft tissue (SSTI). This association of PVL positive *S. aureus* with SSTI might explain the higher level of occurrence with these isolates.

V. Conclusion

This study which set out to explore the virulence potential of *Escherichia coli* and *Staphylococcus aureus* isolated from specific Naira denominations circulating in Rivers State, Nigeria reports on a low occurrence of *S. aureus* virulence genes. For *E. coli* however, rather than the *E. coli* strains being innocuous they were found to have a high association with virulence factors. Furthermore, these genes described in *E. coli* have a known association with mobile genetic elements and hence possess the capacity to transform harmless commensal strains to pathogens.

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