



Urinary Tract Infections in Benin: Exploring the Virulence Factors and Antibiotic Resistance and Virulence Genes among Bacterial Isolates

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Authors' contributions

This work was carried out in collaboration among all authors. Authors VD, PA, JM, JA, ED and KF wrote the protocol, performed the study and designed the manuscript. Authors VD and PA performed the statistical analyses. Authors JD, HB, and LBM reviewed the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Objective: The emergence and increasing spread of resistance to antibiotics in uropathogenic Enterobacteriaceae is a huge public health problem and increase the morbidity and mortality. The objective of this study was to produce scientific data on the virulence and level of resistance of these bacteria in Benin.

Results: This study was conducted on 230 strains of enterobacteria isolated from patients with urinary tract infections. These virulence factors sought were hemolysis, hemagglutination, serum

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resistance, biofilm formation, and the production of lipase, protease and lecithinase. The molecular characterization of the virulence and antibiotic resistance genes was accomplished by PCR according to the different conditions for each gene. The bacteria possessed several types of virulence factors such as hemagglutinin (28.26%), lipase production (23.92%) and hemolysin production (17.83%). The virulence gene identified were FimH (74.79%), PapC (30.44%), Iss (11.34%) and Biofilm (3.92%). The antibiotic resistance genes such as Bla-SHV (49.56%), Bla-CTX-M15, Bla-CTX-M2 (33.92%), Bla-CTX-M9 (19.13%) and bla-CTX-M1 (11.74%) were also detected.

Conclusion: These results filled the national gap about virulence and antimicrobial resistance of enterobacteria responsible of urinary tract infection and may be used to improve the management of patients in Benin.

Keywords: Virulence factors; resistance gene; enterobacteria; urinary tract infections.

1. INTRODUCTION

Urinary tract infection is one of the most frequent bacterial infections in general medicine and most often encountered in both community and hospital settings with a rate of 40% [1]. In United States, this infections accounted for more than 11 million physician visits, 1.7 million emergency room visits and nearly half a million hospitalizations with an estimated societal cost of \$3.5 billion per year [2]. In France, it was reported that 47% of the bacterial infections were urinary tract infections.. Several studies carried out in Africa have shown that urinary tract infections are an endemic disease and that most of the bacteria isolated are multi-resistant [3]. Antimicrobial resistance, has become a major threat to the prevention and treatment of bacterial infections worldwide [4]. According the WHO, more than 700,000 people around the world die each year from antibiotic-resistant infections and, if no action is taken, it is estimated that these infections will kill 10 million people a year in 2050 [5]. Of all the studies carried out on the resistance of bacteria in West Africa, a high level of resistance to commonly used antibiotics has been found. Unexpectedly, in both inpatients and outpatients, a high level of resistance to third-generation cephalosporins has also been observed, to be related to bacteria that produce β -extended spectrum lactamase [6]. Studies carried out in certain West African countries show that in Senegal, this is 21.2%, Guinea-Biseau (36.2%), Mali (63%), Nigeria (27, 8%), Ghana (49%), Benin (35%) and Togo (66%) [7,8,9]. In addition, in Benin, the study of Koudokpon et al. [10] explains that the ESBL producing bacteria are in perfect extension. All these studies carried out in West Africa have addressed the resistance of bacteria isolated from human pathology, but none of them have focused on the virulence and resistance of enterobacteria isolated from urinary tract

infections. These virulence properties enable them to cross host defense mechanisms in order to establish themselves in new ecological niches and to express their pathogenicity [11]. The lack of scientific data on the virulence and the real level of resistance of enterobacteria responsible for urinary tract infections justifies the importance of the present study. The general objective was to produce scientific data on the virulence the of resistance of enterobacteria responsible for urinary tract infections in Benin.

2. MATERIALS AND METHODS

2.1 Biological Material

This study was carried out on 75 *Escherichia coli*, 58 *Enterobacter cloacae*, 60 *Klebsiella pneumoniae*, 18 *Klebsiella oxytoca*, 15 *Enterobacter aerogenes*, 2 *Citrobacter diversus* and 2 *Morganella morganii* Isolates recovered from patients suffering with UTI. Various patient information including socio-demographic and other characteristics, the manipulations results of sample were noted [12].

2.1.1 Phenotypic detection of virulence factors

Virulence factors were investigated according to the methodology described by Hassan et al. [11].

- (A) Haemolysis: The hemolytic property of the isolate was tested using blood agar. The bacteria were cultured on the blood agar and incubated for 18 to 24 hours. The presence of clear halo around the bacterial colony indicated a positive result.
- (B) Haemagglutination: The haemagglutination was detected with the red blood cells from blood group O, washed three times with physiological saline and a drop of the 3% suspension was placed on a microscope slide. A drop of the bacterial suspension in nutrient broth was subsequently mixed.

- The mixture was whirled for 5 minutes at room temperature. The formation clumps or flakes reflected a positive result.
- (C) Serum resistance: 150 microliters of the human serum were mixed with 50 microliters of the bacterial suspension in a 96-well microplate in duplicate. A negative control was done using sterile distilled water. The mixture was initially read in spectrophotometer at 620 nm and 3h after incubation at the same wavelength. If the optical density of the reading after incubation was 100% greater than the initial reading, it is concluded that the bacteria is serum resistant [11].
 - (D) Lipase production: The lipase production was investigated using 1% Tween agar. The bacteria were cultured on agar and incubated at 37°C for 48h to 7 days. The formation of an opaque zone around colonies was a positive result [11].
 - (E) The Protease production was detected with soluble casein agar. The bacteria were cultured on 15% soluble casein agar and studied for 24 hours at 37 °C. The presence of opaque area around the bacterial colony results in a positive result [11].
 - (F) Lecithinase production was investigated using 2.5% egg yolk agar [11]. The bacteria were cultured on this agar and incubated at 37 °C for 7 days. The presence of opaque area around the colony shows a positive result.
 - (G) Biofilm production was studied by the technique of Congo red dye method [13]. The bacteria were cultured on Congo Red agar and incubated for 24 hours at 37 °C. The bacteria having a black colony with a dry crystalline consistency are the biofilm producing bacteria.

2.1.2 Molecular detection of virulence and resistance genes of isolates

This started by the DNA extraction using the Red Qiagen extraction kit according to manufacturer's guidelines. The isolates were tested for different virulent genes and resistance genes using PCR with specific primer pairs. Several quantities of the mix were prepared (Supplementary Table S1).

The amplification of virulence genes (PapC, FimH, Iss and Biofilm) were carried out using the method described by Hassan et al. [11] and the resistance gene (blaCTX-M1, blaCTX-M2, blaCTX-M9, blaCTX-M15, blaTEM, SHV) as per

the method of Memariani et al. [14]. About OXA-48, this was carried out using the method described by Koudokpon et al. [10]. The PCR products were electrophoresed in 2% agarose gel on a stained gel with 5 µg / ml of red gel with a 100 bp DNA ladder as a molecular weight marker. SupplementaryTable S2, Table S3 shown respectively the primers used for the virulence and resistance genes. During this study, a positive control and a negative control was used for each test carried out.

2.2 Statistical Data Analysis

The data collected were entered in the software GraphPad Prism version 7 and the graphs were drawn with this software..

3. RESULTS

The majority of samples were collected from females. The study of antibiotic susceptibility has shown: amoxicillin (50.56%), amoxiclav (91.82%), cefotaxime (36.24%), ceftriazone (45.58%), aztreonam (25, 81%), imipenem (2.00%), ertapenem (36.07%), gentamicin (33.74%) and ciprofloxacin (52.05%) [12]. Also 23.03% bacteria were beta-lactamase producers as per the double synergy test between Amoxiclav and third generation cephalosporins [12].

3.1 Phenotypic Identification of Virulence Factors

The study has detected several types of virulence factors in the isolates (Table 1). 20 strains of *Escherichia coli* studied were detected to produce most virulence factors except for the production of protease. Haemagglutination was the most frequent virulence factor (26.67%) followed by lipase production (21.34%) and haemolysis (20.00%). This was same with the strains of *Klebsiella pneumoniae* and *Enterobacter cloacae*. All the virulence factors sought were found in at least one strain of *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter cloaceae* except for lecithinase production which was absent in all bacterial species studied. In addition, only seven (7) strains of *Escherichia coli* and two strains of *Klebsiella pneumoniae* were positive for biofilm production. Supplementary Fig. S1 indicates the prevalence global of each virulence factor and Fig. S2 shows the image of the positive aspects of the search for different virulence factors on the appropriate agar plates. The Fig. S2-b show the aspect positive of biofilm on agar plate.

Table 1. Prevalence of the various virulence factors identified in the bacterial species studied

Strains	Virulence Factors						
	Lipase	Protease	Lecithinase	Haemagglutination	Hemolyse	Biofilm	Serum Resistance
<i>Escherichia coli</i>	16/75 (21,34%)	00/75(0,00%)	05/75 (6,67%)	20/75 (26,67%)	15/75 (20,00%)	07/75 (9,34%)	14/75 (18,67%)
<i>Klebsiella pneumoniae</i>	14/60 (23,34%)	00/60 (0,00%)	02/60 (3,34%)	19/60 (31,67%)	12/60 (20,00%)	02/60 (3,34%)	01/60 (1,67%)
<i>Enterobacter cloacae</i>	15/58 (25,86%)	00/58 (0,00%)	01/58 (1,72%)	21/58 (36,20%)	05/58 (8,68%)	00/58 (0,00%)	09/58 (15,52%)
<i>Klebsiella oxytoca</i>	06/18 (33,34%)	00/18 (0,00%)	02/18 (11,12%)	02/18 (13,34%)	06/18 (40,00%)	00/18 (0,00%)	02/18 (11,12%)
<i>Enterobacter aerogenes</i>	02/15 (13,34%)	00/15 (0,00%)	01/15 (6,67%)	02/15 (13,34%)	02/15 (12,34%)	00/15 (0,00%)	00/15 (0,00%)
<i>Morganella morganii</i>	1/2 (50,00%)	0/2 (0,00%)	0/2 (0,00%)	0/2 (0,00%)	0/2 (0,00%)	0/2 (0,00%)	0/2 (0,00%)
<i>Citrobacter diversus</i>	1/2 (50,00%)	0/2 (0,00%)	0/2 (0,00%)	½ (50,00%)	1/2 (50,00%)	0/2 (0,00%)	0/2 (0,00%)
Total	55/230	00/230	11/230	65/230	41/230	09/230	27/230

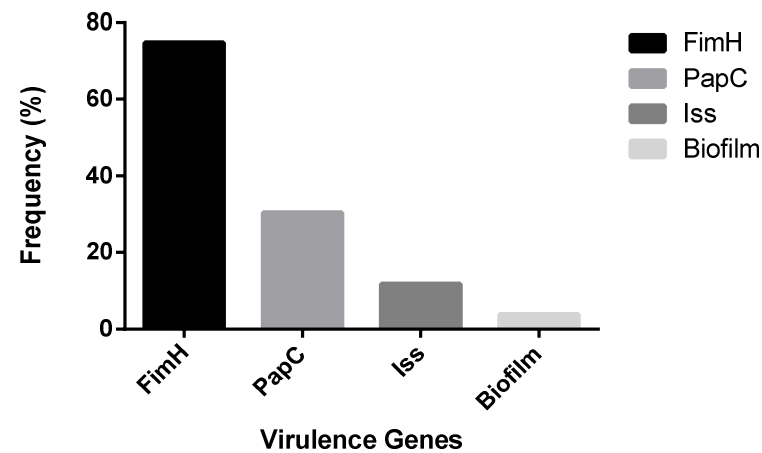


Fig. 1. Prevalence of different virulence genes identified in the isolates ence of the various virulence factors identified in the bacterial species studied

3.2 Genotypic Identification of Virulence and Resistance Genes

The study identified biofilm production gene in total 3.91%, *Iss* in producing, 11.74%, *PapC* gene in 30.43%, and *FimH* gene in 74.78 % of the total bacterial isolates. A total of 9 bacterial strains had the gene for Biofilm production, which is consistent with the phenotypic results. *FimH* was the most identified virulence gene. The analysis of the different results obtained shows that *E. coli* was the bacteria having a high prevalence of different virulence genes studied with the exception of *PapC* gene which was harboured more in *Klebsiella pneumoniae* (78.83%) as shown in Supplementary Table S3; Fig. S3 shows the results of migrations of the virulence genes on the agarose gel.

The proportion of different antimicrobial resistance genes identified shows that *Escherichia coli* and *Klebsiella pneumoniae* are the isolates with the highest proportions of ESBL genes (Table 2). Supplementary Fig. S4, shown a global prevalence of resistance genes. We detected the presence of *Bla-SHV* in a high proportion (49.78%) followed by *Bla-CTX-M15* (33.91%) and *Bla-CTX-M2* (33.91%). However, no gene for carbapenem resistance and *Oxa-48* was identified among the isolates. Supplementary Fig. S5 shows the results of migrations of the resistance genes on the agarose gel.

4. DISCUSSION

The search for virulence factors in the isolates has shown that a large number of strains have several virulence factors. Biofilm provides bacteria with several benefits, such as acquiring antibiotic tolerance, the expression of several virulence factors, and increased resistance to phagocytosis and other host defense mechanisms [15,16,17]. The present study detected 3.91% isolates harbouring biofilm producing gene, majority of which were *E. coli*. This result could also justify the very frequent isolation of *Escherichia coli* strains in cases of urinary tract infections. These results corroborate three studies [11,18,19]. *Escherichia coli* (20%) and *Klebsiella pneumoniae* (20%) are the two bacterial species that produced more hemolysin. The production of haemolysin as a virulence factor in bacteria involved in infections has been demonstrated by other authors in previous studies [20,21]. Lipase production was exhibited

by 23.92% of the bacterial strains evaluated. The lipase-producing bacteria are endowed with catalytic activity which gives these bacteria a virulence factor in human pathology [22,23]. Hemagglutination was recorded in 28.26% of the isolates. This result corroborates the work of Hassan et al. [11]. This factor is partly responsible for the adhesion of the bacteria [24,25,26]. In this study, we also explored the production of protease, lecithinase, and serum resistance. The production of protease among the strains was not detected in this study. Kausar et al., [27] obtained a similar result. Lecithinase was produced at 4.79%. This prevalence is slightly higher than that of Hassan et al. [11]. Raksha et al. [28] also cited lecithinase among the virulence factors of *Enterobacteriaceae*. The pathogenicity of these bacteria is partly a function of their ability to escape the bactericidal effect of serum, which is mediated by the complement cascade. This result is consistent with those of Hassan et al. [11].

The virulence genes identified were *FimH* (74.78%), serum resistance (11.31%), *PapC* (30.44%) and biofilm (3.92%). The detection of *FimH* and *PapC* genes was related to the production of hemagglutinin and hemolysin. These results are consistent with a report by Hassan et al. [11].

The detection for ESBL resistance genes showed that 74.30% of the studied species harboured an ESBL resistance gene. This prevalence was higher than some studies conducted in Benin [7,29]. The prevalence of 74.30% found in the current study simply shows that the bacteria carrying these genes are spreading in developing countries such as Benin. Several authors have also shown that the spread of ESBL genes is rapidly increasing [30,31]. Among the genes identified, there was a high rate of *Bla-SHV* (49.57%) in the strains followed by *Bla-CTX-M* (24.73%). The results confirm the work of others authors [32,33]. This result is also found by several authors who have studied ESBL-producing enterobacteria [34,35]. Among *Bla-CTX-M* identified, *Bla-CTX-M15* (33.92%) and *Bla-CTX-M2* (33.92%) are the most identified. The high prevalence of *Bla-CTX-M15* has been reported in Africa and around the world [36,37,38]. None carbapenem resistance gene has been identified among the isolates. This result is in agreement with the results of the antibiogram which showed a very low level of carbapenem resistance.

Table 2. Distribution of resistance genes in the bacterial strains

Strains	Resistance Genes						
	BlaCTX-M1	BlaCTX-M2	BlaCTX-M9	BlaCTX-M15	BlaSHV	BlaTEM	Oxa-48
<i>Escherichia coli</i>	06/75 (8,00%)	34/75 (45,34%)	20/75 (26,67%)	31/75 (41,34%)	24/75 (32,00%)	00/75 (0,00%)	00/75 (0,00%)
<i>Klebsiella pneumoniae</i>	08/60 (13,34%)	21/60 (35,00%)	09/60 (15,00%)	13/60 (21,67%)	35/60 (58,34%)	00/60 (0,00%)	00/60 (0,00%)
<i>Klebsiella oxytoca</i>	03/18 (16,67%)	10/18 (55,56%)	02/18 (11,12%)	7/18 (38,89%)	10/18 (55,56%)	00/18 (0,00%)	00/18 (0,00%)
<i>Enterobacter cloacae</i>	06/58 (10,34%)	12/58 (20,69%)	12/58 (20,69%)	24/58 (41,38%)	35/58 (60,34%)	00/58 (0,00%)	00/58 (0,00%)
<i>Enterobacter aerogenes</i>	02/15 (13,34%)	01/15 (06,67%)	2/15 (13,34%)	02/15 (13,34%)	07/15 (46,67%)	00/15 (0,00%)	10/15 (0,00%)
<i>Morganella morganii</i>	1/2 (50,00%)	0/2 (0,00%)	1/2 (50,00%)	0/2 (0,00%)	2/2 (100%)	0/2 (0,00%)	0/2 (0,00%)
<i>Citrobacter diversus</i>	1/2 (50,00%)	0/2 (0,00%)	0/2 (0,00%)	1/2 (50,00%)	1/2 (50,00%)	0/2 (0,00%)	0/2 (0,00%)
Total	27/230	78/230	44/230	78/230	114/230	00/230	00/230

5. CONCLUSION

The bacteria have several virulence factors and resistance gene responsible for their degree of pathogenicity and resistance that explain the frequency of identification of these *Enterobacteriaceae* in this infection.

6. LIMITATIONS

This study is limited by the method used to determine the production of biofilm by bacteria.

AVAILABILITY OF DATA AND MATERIAL

All data generated or analyzed during this study is included in this published article and supplementary information files.

CONSENT AND ETHICS APPROVAL

The study has been submitted to the Benin National Ethical Committee for Health Research. An approval has been issued under the number N°65/MS/DC/SGM/DRFMT/CNERS/SA. The approval letter is available upon requested from the corresponding author. The respondents gave their verbal consent to participate in the study.

SUPPLEMENTARY MATERIALS

Supplementary material is available in the following link: <https://www.ijpr.com/index.php/IJPR/libraryFiles/downloadPublic/2>

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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