Enterobacteria responsible for urinary infections: a review about pathogenicity, virulence factors and epidemiology

Victorien Dougnon[1*], Phénix Assogba[1], Eugénie Anago[1], Esther Déguénon[1], Christina Dapuliga[2], Jerrold Agbankpè[1], Septuce Zin[1], Remi Akotègnon[1], Lamine Baba Moussa[1], Honoré Bankolé[1]

[1]Research Unit in Applied Microbiology and Pharmacology of Natural Substances, Research Laboratory in Applied Biology, Polytechnic School of Abomey Calavi, University of Abomey Calavi, Benin
[2]Microbiology Department, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

ARTICLE INFO

ABSTRACT

Urinary tract infections (UTIs) are one of the most common types of bacterial infections in humans, both in the community and in health care settings. UTIs include a range of clinical entities ranging in severity from asymptomatic infection to acute cystitis, prostatitis, pyelonephritis, and urethritis. This is one of the most common diseases encountered in medical practice today, affecting people of all ages, from newborn to geriatric age group. The bacteria most commonly implicated as agents responsible for UTIs generally originate in the intestine and include *Escherichia coli*, *Pseudomonas* spp., *Streptococcus* spp., *Proteus* spp., *Klebsiella* spp., *Staphylococcus* spp., *Neisseria gonorrhoeae, Chlamydia trachomatis*, and *Candida* spp. There are virulence factors of urinary pathogens that promote adhesion to mucosal surfaces and subsequent infections. The multiresistant of these enterobacteria responsible for UTIs is a major public health problem. Antibiotic resistance remains a major problem, especially in the developing countries where hygiene conditions are still precarious and antibiotic use is often abusive and poorly controlled. The multidrug-resistant Enterobacteriaceae most implicated in UTIs by extended-spectrum beta-lactamases (ESBL) and carbapenemase production are: *Klebsiella pneumoniae*, *E. coli*, and *Proteus* spp., not to mention *Acinetobacter baumanii* and *Pseudomonas aeruginosa*, the most carbapenemase producing. The detection of ESBL and carbapenemase production is based mainly on phenotypic and genotypic tests.

1. INTRODUCTION

Urinary tract infections (UTIs) remain a global challenge, especially in sub-Saharan Africa [1,2]. Every year around the world, about 150 million people are diagnosed with UTI, which costs the global economy more than $ 6 billion [3]. In 2006, in the United States, UTIs resulted in 11 million medical visits and 500,000 hospitalizations at a cost of $ 3.5 billion [4]. Epidemiological statistics from African countries are alarming. UTI is the most common bacterial infection in humans infecting largely sexually active individuals. Although this disease is sex-related, sex individuals are the most infected with this bacterial infection. Women are more susceptible to UTI than men. About 50% of women experience UTI in their lifetime [5].

Enterobacteria account for more than 90% of bacteria isolated in cases of UTIs. Also, enterobacteria are part of the normal intestinal flora of humans unlike other bacterial species and parasites. In addition, enterobacteria are the bacterial species most implicated in human infections in recent years, including UTIs. This very high proportion depends on the interaction of enterobacteria with infected tissues [6]. This interaction can start from a simple adherence to invasion and cell lysis. These interactions reflect the synthesis by microorganisms of various types of active substances and condition the pathogenicity [7]. The pathogenicity of these bacteria is dependent on the presence of multiple virulence genes such as operative adhesins through fimbriae, O antigens, K capsular antigens, serum resistance, hemolysis production, and others. UTI can be treated with antibiotics; however, multiple resistance mechanism of bacteria against antibiotics is a threat to the management of UTI.

Corresponding Author
Victorien Dougnon, Research Unit in Applied Microbiology and
Pharmacology of Natural Substances, Research Laboratory in Applied
Biology, Polytechnic School of Abomey-Calavi, University of Abomey-
Calavi, Benin. E-mail: victorien.dougnon@gmail.com

The discovery of antibiotics has been a success for the treatment of bacterial diseases. The uncontrolled and uncontrolled use of these antibiotics allowed the bacteria to develop antibiotic resistance very quickly which is today a global public health problem [8].

Several studies conducted around the world have mainly incriminated Gram-negative bacilli, which are the ones that develop the most resistance mechanisms [9]. The latter expresses a high level of acquired resistance to the majority of beta-lactam antibiotics by the production of enzymes called extended-spectrum beta-lactamases (ESBL) inactivating first-, second-, and third-generation cephalosporins [10]. Bacterial resistance also concerns families of antibiotics, such as carbapenems, penicillins, aminoglycosides, quinolones, and others. The genes encoding these enzymes are plasmid-borne and coexist with genes for resistance to other antibiotics, hence the origin of the multi-resistance of extended-spectrum beta-lactamase-producing enterobacteria [11]. This multi-resistance of enterobacteria to betalactamines led to the prescription of carbapenems in clinical trials, a last resort in the pharmaceutical factory. The introduction of carbapenems favored the production of carbapenemases, an enzyme produced by bacteria that inactivates these antibiotics. [9]. This multi-resistance problem of Enterobacteriaceae is more noticeable in the developing world and worse in Benin country where the situation of the prevalence, virulence, and multiresistance of these Enterobacteriaceae responsible for bacterial infections including UTIs is not mastered.

The general objective of this paper is to provide through scientific journals, information on the pathogenicity, virulence, and multiresistance of enterobacteria responsible for UTIs [9].

2. MATERIALS AND METHODS

Review work was conducted using the following keywords: Enterobacteria, Multiresistance of Enterobacteriaceae, Involvement of Enterobacteriaceae in UTIs, Enterobacteria Producing Spectral Beta-Lactamases. expanded', "Enterobacteria producing carbapenemases." These keywords were entered into the search engines of Google Scholar, PubMed, Researchgate and the articles were used according to the reliability of their source, the study area, and the subject.

3. WHAT IS UTI?

UTI is a microbial proliferation accompanied by an inflammatory reaction in the urinary tract. It can be in the form of urethritis, cystitis, or pyelonephritis [12]. Globally, it is defined by the presence of the bacteria in large number (greater than 10^5 bacteria/ml) in the urinary tract and/or the renal parenchyma. Therefore, the concept of a UTI is broad, ranging from asymptomatic bacteriuria to pyelonephritis with sepsis [13].

UTIs rank second among infectious diseases contracted by man after respiratory diseases [13]. These infections are characterized by a rather high frequency in women than in men because of the conformation of the female urogenital system [14]. UTI is an infection of both the container (urinary tract) and the contents (urine) [16]. It is thus an inflammation sometimes localized to the excretory ways (pyelonephritis-cystitis), sometimes also concern the renal parenchyma (pyelonephritis) [15]. Several bacterial species are responsible for UTIs. In view of the epidemiological data and the etiology of the bacteria responsible for UTIs, Enterobacteriaceae are the most represented [16].

4. WHAT ARE ENTEROBACTERIA?

4.1. Definition and Description of Enterobacteriaceae

The Enterobacteriaceae family is defined by the following features: Gram-negative bacilli (2–4 microns long by 0.4–0.6 microns wide), mobile with peritrichous ciliature or immobile, growing on ordinary culture media, aerobic—facultative anaerobes, fermenting glucose with or without gas production, reducing nitrates to nitrites, and negative oxidase [17].

Enterobacteria are a very heterogeneous family in terms of pathogenesis and ecology. The genus that make up this family are indeed either parasites, commensals (*Escherichia coli*, *Proteus mirabilis*, and *Klebsiella* spp.), or even saprophytes (*Serratia* spp., *Enterobacter* spp.). [17].

Several studies have reported that these bacteria are involved in the majority of hospital and community infections, especially UTIs. The most common microorganisms in patients with UTI are called uropathogens [15]. Most infections of the urinary tract are due to the upward propagation of intestinal bacteria resulting in the predominance of enterobacteria in which:

- *Escherichia coli* (is most often involved, 60%–80%)
- Proteus (*P. mirabilis*, *Proteus vulgaris*, and *Proteus rettgeri*)
- Klebsiella (*Klebsiella pneumoniae* and *Klebsiella oxytoca*)
- Enterobacter (*Enterobacter cloacae* and *Enterobacter aerogenes*)
- *Providencia stuartii* and *Morganella morganii*

Pseudomonas aeruginoa species are also found in iatrogenic UTIs resulting from the application of medical devices [18]. The virulence and pathogenicity of these bacteria responsible for human infections including UTIs vary.

4.1.1. Pathogenicity

The normal urinary tract is sterile above the distal third of the urethra, which has a mixed commensal flora [19]. Infection develops only when bacterial virulence overcomes the normal defense mechanisms of the host. We have the natural defense mechanisms such as the walls of the urinary tract, non-specific immunological mechanisms such as phagocytic cells, and finally the mechanisms of specific immunological defenses such as lymphocytes and others. *Escherichia coli* is the most common uropathogen in the community and in hospitals. The different forms of manifestation of UTIs are as follows:

Cystitis—This is the most common form of UTIs [19]. It manifests as dysuria, suprapubic pain, hematuria, and offensive or cloudy urine. Typical symptoms, when present, are sufficient to diagnose cystitis. Systemic symptoms such as fever, nausea, and vomiting may occur [20].

Pyelonephritis—Infection with the renal parenchyma is usually due to ascending infection of the bladder. In addition to local

symptoms (loin pain, hematuria, and possibly cystitis symptoms (50% of cases), symptoms and systemic signs are frequent and often severe, with fever, vomiting, and septic shock. Often high and blood cultures are positive in 20% of cases [21].

Although the infection is usually focal, a diffuse infection that may cause kidney failure may occur. Severe infection can cause papillary necrosis, especially in patients with diabetes mellitus, who are also particularly susceptible to emphysematous pyelonephritis if the infection is caused by a gas-producing organism (often *E. coli*, facultative anaerobic). It is a life-threatening infection that often requires nephrectomy. An abscess, within the kidney or beyond the renal capsule (perineal abscess) may form. This must be taken into account if the symptoms and signs of infection do not disappear with appropriate antibiotic treatment [21].

Urethritis—It can be manifested by dysuria and discharge from the urethra. It is primarily a sexually transmitted disease caused by *N. gonorrhoeae, C. trachomatis, Mycoplasma genitalium*, *or Trichomonas vaginalis.*

Prostatitis—It can be acute or chronic depending on the duration of symptoms and is the most common recurrent UTI in men. It usually presents with perineal or scrotal pain, frequency, urgency, and dysuria [22].

Not all species of bacteria are identical in their ability to induce an infection. This capacity depends on factors related to the host and the bacterium (virulence): when the natural defenses of the organism are diminished, it is not necessary that the strain of microbes is virulent to trigger the infection; some strains of bacteria, in the same species, have virulent factors allowing them to go up from the fecal flora, the vaginal milieu, or the periurethral space, to the urethra and the bladder, or less frequently until kidneys, thereby inducing systemic inflammation [23].

Among the very few antigens that enterobacteria possess, only O, H antigens and envelope antigens (Vi, K, etc.) are used for diagnosis. They are generally sought by agglutination using a technique that has the advantage of being simple, fast, and accurate enough [24].

To overcome the flow of urine, uropathogenic bacteria express a series of fimbrial adhesions. Fimbriae are filamentous structures that protrude from the surface of the bacteria and are responsible for binding to the epithelium of the host. Uropathogens also produce toxins, hemolysin, and colony necrosis factor, which disrupt epithelial integrity and allow bacterial invasion.

The colonization of the urinary tract, generally defined by $>10^5$ bacteria/ml in freshly emptied urine, gives rise to a panoply of clinical manifestations. UTIs, especially in children and the elderly, may not show typical symptoms and the diagnosis can easily be omitted [25].

Adhesion to solid substrates is a property common to many pathogenic microorganisms, including viruses, Gram-positive and negative bacteria, yeasts, and protozoa [26]. By binding to host structures, microbial pathogens avoid being swept away by the normal flow of body fluids (blood, urine, and intestinal contents) and eliminated [27], although host cells containing adherent bacteria can be removed, thus eliminating organisms despite

attachment [27]. Fixation is considered a necessary first step in the colonization of the mucosal surfaces of the host and a precedent of invasive infection in many situations [27].

4.1.1.1. Fimbriae

Fimbriae (pili) are filiform structures emerging from the bacterial surface. They were described for the first time by Anderson (1949) [28] and thoroughly reviewed by Elhani et al. [28] who also wrote several papers on the serology of fimbrial antigens and their ability to cause direct hemagglutination. Fimbriae are found in all genera of Enterobacteriaceae. Their development depends on growth conditions. They thrive better in fluid cultures and fimbriation is often associated with film formation; however, they are often well developed on ordinary agar media.

A more systematic examination of the antigenic properties of Enterobacteriaceae fimbriae has not been performed, but it is clear that there are several fimbrial antigens among Enterobacteriaceae and many cross reactions between fimbriae of different genera. The same strain may have several fimbrial antigens simultaneously.

Previous studies have shown that many Enterobacteriaceae isolates from the urinary tract and gastrointestinal tract express type 1 fimbriae [28].

4.1.1.2. Antigen O

This lipopolysaccharide antigen is thermostable at 100°C. This antigen comprises three parts: the lipid wall, the "core" part, and the polysaccharide [27].

4.1.1.3. Capsular polysaccharides (K antigen)

Capsular and microcapsular polysaccharides are present in many genera of the family Enterobacteriaceae. However, capsules with the same similarities can be found in bacteria of the same family: for example, *E. coli*, *Enterobacter* spp., and *Serratia* spp. The term capsule is most often used to designate an external substance that covers the entire surface of the bacterium and is so strongly bound to other surface structures that it is not completely solubilized in a fluid medium [26].

The most frequently used method for the determination of K antigen is the Quellung reaction [38], also known as the Neufeld reaction, a biochemical reaction in which antibodies bind to the bacterial capsule of *Streptococcus pneumoniae, K. pneumoniae, Neisseria meningitidis, Bacillus anthracis, Haemophilus influenzae, E. coli, and Salmonella.* The reaction of the antibodies makes it possible to visualize these species under a microscope. If the reaction is positive, the capsule becomes opaque and appears to be enlarged [25].

The protocol has three main steps, namely, the preparation of a bacterial cell suspension, the mixing of these cells and antisera on a glass slide, and the reading of the Quellung reaction using a microscope [27].

By adding a specific antiserum to the K (capsular) antigen, a precipitation reaction occurs at the bacterial surface. This makes the capsule highly refractive and therefore easily visible under a

microscope. Ink-based preparations from China are recommended to determine the size of the capsule [29].

All these virulence factors contribute to the degree of pathogenicity of enterobacteria and their multidrug resistance, especially through the production of biofilm. Other virulence factors are hemolysis. Protease production, hemagglutination, serum resistance, lipase production, and lecithinase production are all other virulence factors in enterobacteria and also contribute to the resistance of these bacteria to antibiotics.

4.1.2. Epidemiology

The urinary tract is one of the body's sites most infected by bacteria, but this frequency varies with age and gender.

UTIs in children are common. 2% of children have a UTI before the age of 10 [30]. The prevalence of UTIs is 3% in girls and ranges from 1%–2% in boys. However, during the neonatal period, boys are more affected than girls (sex ratio = 2.5), whereas beyond 1 year, the UTI reaches three times more in girls than of boys, with a peak between 2 and 3 years [31].

Rapid diagnosis, investigation, and treatment of UTIs are necessary to minimize the risk of renal scarring. Children with a combination of infection and urine reflux from the bladder into the ureter and kidneys are at particularly high risk for renal injury (reflux nephropathy) [25].

The literature indicates that urinary infections after 1 year (and even in adults) predominate in girls [26]. The anatomical reason for this is because the girl's urethra is short and close to the perianal region, which often causes cystitis.

UTIs during pregnancy can occur in 20% of pregnancies and are associated with an adverse outcome. Asymptomatic bacteriuria occurs in the first trimester in 29% of pregnant women and a symptomatic infection will develop in 30% of them.

UTIs in urology patients are common. Bacteriuria occurs in 5% of patients with urinary catheters per day and even short-term catheterization is associated with a 10% risk of UTI. Any structural abnormality predisposes to UTIs, which may be recurrent and require long-term prophylactic antibiotic therapy.

UTIs in kidney transplant recipients are the most common infection at the beginning of the post-transplant period and are more likely to result from abnormal anatomy, catheterization, and ureteral stenting than immunosuppression. Acute pyelonephritis in a grafted kidney should be treated promptly as it may result in scarring in the grafted kidney [32].

5. BACTERIOLOGICAL DIAGNOSIS OF URINARY TRACT INFECTIONS

The Cytobacteriological examination of urines makes it possible to demonstrate the presence or absence of a urinary infection in a patient [32]. This examination is carried out in several stages.

5.1. Sample

Urine sampling must be performed after careful personal hygiene to avoid any risk of disruption of results. The urine should be collected in a sterile vial provided for this purpose and issued by the testing laboratory. It is strongly recommended to collect the urine of the morning, preferably the second jet in order to evacuate the bacteria present in the urethral meatus [33].

For women, Cytobacteriological Urine Exam should, as far as possible, be performed outside the menstrual period. If the sample is not taken in the laboratory, it must be brought within 2 hours. In the meantime, it must be stored in a refrigerator at + 4°C to prevent germs from proliferating. In the newborn or child who is not yet clean, the sample is taken via a collecting bag [34].

5.1.1. Examen macroscopic

The urine can be limpid, cloudy, mahogany, straw yellow, herbal; contain filaments, deposits, or colored as a result of taking certain medications. The pathological urines are usually turbid. The normal urine is limpid, straw yellow, pH between 5 and 6. However, clear urine can be pathological, especially in case of polyuria or in immunocompromised subjects [35].

5.1.2. Microscopic examination

This is an important step in ECBU. It includes a fresh examination and an examination after Gram stain. Fresh examination includes leukocyte count and urinary pellet examination.

Leukocytes are counted on total urine using a counting cell (Malassez,). The result is expressed in leukocytes/mm^3. Normal urine contains less than 10 leukocytes/mm^3. In case of infection, this value increases and exceeds the threshold of 10 leucocytes/ mm^3 [35].

The examination of the urinary pellet allows a semi-quantitative evaluation of the figured elements contained in the urine. They are: leucocytes, red blood cells, epithelial cells (renal round, racquet of vesicle origin, and large cells with small nuclei of vaginal origin), crystals (calcium oxalate, urates, phosphates, etc.), cylinders (hyaline, granular, and leucocytic, fatty), yeasts, parasites (*T. vaginalis* and *Schistosoma haematobium* eggs), and bacterial flora (morphology, abundance, and mobility) [35].

Stain examination consists of Gram stain. It allows to differentiate the two types of Gram: Gram-positive or Gram-negative. At this stage, we recognize the real morphology of bacteria (Cocci or bacilli). This examination also provides information on the abundance of bacteria (absence, rare, scanty, abundant) and also on the mode of grouping bacteria (dispersed, clusters, chains, by two and others). Following microscopic examination, culturing is based on the results of Gram stain. The different culture media are therefore chosen according to the type of Gram (Gram-positive or Gram-negative) and the type of bacteria (cocci or bacilli) [36].

5.1.3. Culturing

The bacterial culture takes into account the results of Gram staining. Various cultural backgrounds have been used for this purpose. There are non-selective media in which bacilli and cocci grow: Trypto-casein soy agar, Bromo-cresol-infant agar, agar cystine-lactose electrolyte deficient, and others. Common selective media are: methylene blue Eosin for enterobacteria and Chapman agar for *staphylococci*. The culture plates are placed in an oven

for 18–24 hours of growth. The culture is made using a loop and the urine is spread on the agar either by the French method or the English method. Bacterial growth is observed the next day by a bacterial colony visible to the naked eye on the agar [37].

5.1.4. Isolation and identification

The day after cultivation, a Gram control is performed before isolation. Isolation involves transferring an isolated colony to obtain pure colonies of the bacterial species. The identification of the bacteria will be carried out on the pure colonies. The identification of the bacteria is based on its morphological, cultural, and biochemical characters. In the case of Gram-negative bacilli, modern galleries (API 20E or Leminor's classical gallery) are used [38].

5.1.5. Antibiogram

The antibiogram allows to study the *in vitro* sensitivity of the bacterial strain to usable antibiotics and to adapt the treatment. Two methods can be used but only the diffusion method on agar medium is the commonly used [39].

The study of the sensitivity of bacteria to antibiotics is necessary for better management of patients. To do this, a 0.5 Mc Farland scale bacterial suspension is made of a colony isolated from the bacterial strain. This suspension is cultured on Mueller Hinton agar by the depletion technique with very tight streaks. The antibiotic disks are deposited on the surface of the agar a few minutes later. The disks are arranged at three centers of one and the other for easy reading. The result is read after 24 hours of incubations in the oven. The diameters of inhibition are read and compared to the standard to determine the sensitivity or not of the bacteria. The results are classified into three categories: sensitive, resistant, and intermediate [39].

The choice of antibiotics for the treatment of UTI is therefore based on the results of the antibiogram.

Antimicrobial resistance is one of the major health problems in human and animal medicine [40]. The emergence and spread of antibiotic resistance pose a real threat to global public health. Recent bibliographic data are full of descriptions of multidrug-resistant or even antibiotic-resistant bacteria that continue to grow in both industrialized and developing countries [40]. The situation is alarming in resource-poor countries where infectious diseases, poverty, and malnutrition are endemic. The emergence of antibiotic resistance is a complex process that often involves host factors, environmental factors, and the pathogen [40]. In West African countries, the endemicity of respiratory infections, bacterial meningitis, diarrhea, and other infectious diseases have increased antibiotic consumption for both symptomatic and prophylactic use. In addition, particularly in terms of human resources and/or diagnostic capacity combined with unregulated access to antibiotics contribute to the development of bacterial resistance [40].

6. RESISTANCE OF ENTEROBACTERIA

6.1. Resistance by ESBL Production

β-lactams are a major family of antibiotics that are widely used clinically because of their broad spectrum of action, low toxicity, efficacy, and for low cost. The intensive and often abusive use of these antibiotics was quickly followed by the appearance of multiresistant strains of bacteria and compromised, in many cases, the use in antibiotic therapy of these drugs of choice. Indeed, the bacteria have developed different mechanisms of resistance to β-lactams, including the synthesis of enzymes (β-lactamases) that catalyze efficiently and irreversibly the hydrolysis of the amide bond of the β-lactam cycle giving a biologically produced product inactive [39].

6.1.1. Definition of ESBL

ESBLs are characterized by high-level resistance to amino-carboxy-acylureidopenicillins as well as to first- and second-generation cephalosporins, and by a more or less clear decrease in the activity of and fourth-generation cephalosporins and aztreonam [41]. ESBLs have been so named to differentiate them from their parent enzymes "broad-spectrum beta-lactamases" that do not hydrolyze third-generation cephalosporins (C3G). This name of ESBL thus refers to a broadening of the inactivation spectrum vis-à-vis the C3G or aztreonam. The activity of cephamycin and imipenem remains unchanged. However, these betalactamases are inhibited by beta-lactamase inhibitors (clavulanic acid, sulbactam, and tazobactam) which largely restore the activity of the penicillins with which they are associated. This is a phenomenon of irreversible inhibition with the destruction of the inhibitor (suicide action): the inhibitor serves to decoy the enzyme and is destroyed in place of the antibiotic molecule. In the case of high beta-lactamase production, these may not all be destroyed by the inhibitor, leading to therapeutic failure [41].

6.1.2. ESBL mechanism of action

In the Enterobacteriaceae family, the predominant β-lactam resistance mechanism is enzymatic inactivation by β-lactamase production, particularly ESBL. The latter are inactivation enzymes whose substrates are β-lactams. They are able to open the β-lactam ring by creating an unstable acylenzyme intermediate, ultimately leading to the loss of a carboxyl group that is responsible for the inactivation of the antibiotic [41].

The lack of routine surveillance in most countries and in the West African region does not allow a good estimate of ESBL proportions among strains isolated during infectious processes. However, some studies report the reality of the problem. In Ghana, half of the enterobacteria (49.4%) isolated from the various infections diagnosed at Korle-Bu Hospital were ESBL-producing [18]. Although the prevalence reported in Nigeria is relatively lower (10%–27%) [40], the prevalence of ESBL-producing enterobacteria in the countries of the region remains, in general, very worrying. In Benin, the proportion of *E. coli* strains is estimated at 35%. coli responsible for nosocomial infections producing ESBL. In Togo, 66% of *E. coli* strains in UTIs had the ESBL phenotype [41].

6.1.3. ESBL genes circulating in West Africa

In all the studies cited, Cefotaxim (CTX) M-15 is the most common enzyme found in both circulating strains in the hospital environment and in the community. The emergence

and spread of ESBLs in West African countries are therefore linked to the global expansion of the CTX-M-15 type [42]. Among other CTX-M ESBLs, CTX-M-14 was found in Mali, CTX-M-3 in Nigeria and Senegal. The other enzymes that have been described are SHV-3 and SHV-12, which have appeared in recent years and have been detected in various isolates from Mali and Nigeria. All these plasmids carrying the ESBL gene also harbor other resistance genes that confer the vast majority of ESBL Enterobacteriaceae resistance to other families of antibiotics, including cotrimoxazole, fluoroquinolones, and aminoglycosides [42].

6.1.4. ESBL detection

The detection of ESBL-producing bacteria in the clinical microbiology laboratory is an absolute necessity for proper treatment of patients, appropriate control and prevention of these infections, and monitoring of these multiresistant strains at the level of surveillance systems. The phenotypic expression of ESBL remains, in the majority of cases, a rapid basis for their identification. However, only the molecular approach will allow real individualization [mainly polymerase chain reaction (PCR) and sequencing] [42].

6.1.5. Phenotypic detection

The phenotypic detection of ESBLs is based on the fact that they are inhibited by clavulanic acid. Thus, an increase in C3G activity in the presence of clavulanic acid indirectly indicates the presence of ESBL. Several tests are known and the most used in clinical microbiology is the double synergy test (TDS).

6.1.5.1. The double synergy test

TDS consists of placing a 30 g disk of C3G (cefotaxime, ceftazidime) and/or aztreonam at a distance of 20 to 30 mm, center to center, of an amoxicillin-clavulanic acid disk (10 g). The production of ESBL results in very characteristic synergistic images between C3G or aztreonam and clavulanic acid (Augmentin or Claventin disk): a so-called champagne cork image [40]. It can be noted that some ESBLs are characterized by a low activity with respect to C3G. In this case, the resistance level is low and the synergy images are more discrete. It would then bring the disks closer to observe a synergy. A distance of 15 mm between the disks recommended by some authors would offer a higher sensitivity [38].

6.1.5.2. Combined disks

Combined disks were developed to quantify the synergy between broad-spectrum cephalosporins and clavulanic acid. The principle of this technique is to measure the zone of inhibition around the disk of the cephalosporin alone and that around the disk of the same cephalosporin supplemented with clavulanic acid. A diameter difference greater than 5 mm indicates the presence of an ESBL. This test is easy to do and its interpretation is simple. Its sensitivity and specificity were estimated, respectively, for Klebsiella spp., *E. coli*, Citrobacter spp., and *P. mirabilis* at 100% and 86% using the results of the combined disks (clavulanic acid and cefotaxime, clavulanic acid and cefepime) [40].

Several other tests make it possible to search the ESBLs: the *E*-Test and Cica-Beta-Test. The *E*-test strips, made of plastic, are impregnated with an antibiotic according to an exponential concentration gradient (in g/ml) which makes it possible to obtain, by simple reading, the value of the MIC of an antibiotic for a given bacterial strain. They have been modified to quantify the synergy between broad-spectrum cephalosporins and clavulanic acid. Cica-Beta-Test allows rapid ESBL detection (in 15 minutes) from a 24-hour bacterial culture. This test uses four strips: one strip containing no inhibitor, another containing clavulanic acid (ESBL detection), a third containing boronic acid (detection of AmpC type enzymes), and a fourth containing ethylene-diamine-tetra-acetic (detection metallolactamases). The test is based on the use of a chromogenic cephalosporin, HMRZ-86, which has a protective group that prevents the breakdown of its lactam cycle [40].

6.1.6. Prevalence of ESBL

ESBL prevalence rates vary widely by geographic location, bacterial species, and source of isolates [37]. The prevalence reported in the literature mainly concerned the two species *E. coli* and *K. pneumoniae*. The lowest prevalence (less than 10%) were noted in Northern Europe, Canada, the United States, Japan, Australia, and New Zealand [39]. In contrast, the highest prevalence were reported particularly in Latin America followed by Southern and Eastern Europe. High prevalence rates have also been noted in Africa, particularly in North Africa and South Africa [39]. However, very low prevalence have been noted in some countries in sub-Saharan Africa that probably do not represent the true incidence of ESBLs in this region because the detection of this resistance is not used as a routine test in clinical laboratories. This is the case of Benin [39].

6.1.7. Resistance by Carbapenemase production

Carbapenem-resistant Enterobacteria or carbapenemase-producing Enterobacteriaceae are Gram-negative bacteria resistant to the class of carbapenem antibiotics, considered as a last resort for the treatment of human bacterial infections. They are resistant because they produce an enzyme called carbapenemase that disables the active ingredient of the antibiotic resistance to carbapenems [43].

Carbapenems are β-lactam bactericides with proven efficacy in severe infections caused by ESBL producing bacteria. There are some examples, namely, imipenem, meropenem, doripenem, ertapenem, panipenem, and biapenem, used worldwide because of the increasing resistance to cephalosporins in the Enterobacteriaceae group. Recent new resistance mechanisms accumulate through the spread of carbapenem β-lactamase destruction leaving the therapeutic options narrow [43]. The search for carbapenems was initially from various sources. Of these carbapenems, the selection for treatment depends on the present pathogen.

6.1.7.1. Mechanism of action of carbapenems

Carbapenems are potent members of the β-lactam family that are structurally related to penicillins. The mode of action of carbapenems is first initiated by penetrating the bacterial cell wall and binding to enzymes called penicillin-binding proteins [43]. The main series of protein binding penicillins inhibition are

1a, 1b, 2, and 3; and the resulting lethal effect is the inactivation of an autolytic enzyme inhibitor within the cell wall that leads to the death of bacteria [43].

6.1.7.2. Molecular classification of carbapenemases

A large variety of carbapenemases have been identified in enterobacteria belonging to three classes of β-lactamases: A-class Ambler, B and D β-lactamase. These classes are of greater clinical importance in nosocomial pathogens. Classes A, C, and D β-lactamases all share a serine residue in the active site. The clinical role of a fourth class (Ambler class C) is unknown but has prolonged activity toward carbapenems [43].

6.1.7.3. Detection of carbapenemase production

The presence of a carbapenemase can be detected by a number of methods in clinical laboratories. These include automated systems or disk diffusion, inhibitory minimal concentration, selective agar, modified Hodge test (MHT), synergy tests (e.g., *E*-tests or double disk tests), spectrometry, genome sequencing, and molecular methods. The majority of genes controlling carbapenemase production are transferable by plasmids. The presence of carbapenemase means some relevance to clinicians. However, care should be taken in the management of patients on carbapenem treatment which may vary depending on the resistance mechanism existing at that time. Currently, enzyme detection is difficult because of the different mechanisms involved and unreliable techniques used in some clinical laboratories [43].

6.1.7.4. Phenotypic detection

The basic test that predicts carbapenemase production first is disk diffusion or the use of automated systems. Before this sensitivity test, an identification of the bacterial species is necessary, which takes about 2 days for common bacterial pathogens, and although it is very precise, there are inherent problems in the differentiation between acquired and intrinsic resistance. [43]. For disk diffusion, the impregnated disks containing a standard amount of an antibiotic agent are placed on an agar plate seeded with a bacterium to be tested. As the bacteria grow overnight, the antibiotic diffuses into the agar medium. The sensitivity of the strain tested is proportional to the zone of inhibition produced by the antibiotic used. With automated systems, the instruments are used to analyze antibiotic susceptibility tests with standardized inoculum for the strain tested and diluted in a specialized broth with a decrease in the added antimicrobial susceptibility test indicator. The turbidity of the final inoculum is adjusted to 0.5 standard McFarland.

6.1.7.5. Genotypic detection

Molecular techniques have become an effective tool for the detection of carbapenemase. The chain polymerization reaction performed on colonies can give results within 4–6 hours with excellent sensitivity and specificity and this can reduce the risk of bacteria spreading in hospitals. Wang et al. reported a real-time PCR assay with 100% specificity and sensitivity compared to 90% Klebsiella pneumonieae producing carbapenemase phenotypic activity when evaluated by the MHT and sequencing. More recently, multiplex PCR techniques for the detection of several carbapenemase genes in one assay have been produced. These are mainly focused on the detection of genes in enterobacteria, including their subgroups of carbapenemases. The limit of these modern tools does not have sequence similarity of the genes already described [43].

7. CONCLUSION

UTIs are a public health problem and affect the urinary tract such as the parenchyma, urethra, bladder, and kidneys. Cystitis, urethritis, pyelonephritis, and prostatitis are complications of UTIs in the affected urinary tract. These infections are mainly of bacterial origin, caused mainly by Enterobacteriaceae including *E. coli* (at the head of the line) followed by *K. pneumoniae, Proteus* spp., *Pseudomonas* spp. These bacteria have virulence factors such as O, H, K antigens, fimbriae that increase their pathogenicity in UTIs. The correct identification of the etiologic agent goes particularly through the detection of the antigens for a good diagnosis of the infection by the doctor. Misdiagnosis contributes to poor treatment favoring the emergence of multidrug-resistant strains.

UTIs due to multidrug-resistant enterobacteria are currently a major public health problem. From the economic and social point of view, they have serious consequences both for the high cost of treatment and for the mortality they induce. Multidrug-resistant enterobacteria have several pathogenicity factors that give them more virulence and thus allow them to bypass the defense mechanisms of their hosts. Multidrug-resistant UTI surveillance systems must perform well in order to provide recent epidemiological data, but the multidrug resistance of enterobacteria must be more subject to mathematical modeling in order to have estimates of their prevalence and hence for better prevention and for more effective treatment. A good knowledge of the ecology of multiresistant enterobacteria is, therefore, the first step in the fight against these strains. However, it is also necessary to strictly regulate the use of antibiotics and in addition to this, to look for new antimicrobial molecules insensitive to the action of ESBLs and carbapenemases by plant extracts.

REFERENCES

1. Foxman B. Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. Infect Dis Clin North Am 2014;28:1–13
2. Lavigne JP, Le Moing V, Sotto A. Quels antibiotiques utiliser en pratique courante dans les infections urinaires communautaires en France ? Spectra Biologie 2005;146:18–23.
3. Nielubowicz GR, Mobley HL. Host-pathogen interactions in urinary tract infection. Nat Rev Urol 2010;7:430–41.
4. Agence française de sécurité sanitaire des produits de santé. Recommandations de bonne pratique: diagnostic et antibiothérapie des infections urinaires bactériennes communautaires chez l'adulte, Progrès FMC, 2008, F109-F120.
5. Naber KG, Schito G, Botto H, Palou J, Mazzei T. Surveillance study in Europe and Brazil on clinical aspects and Antimicrobial Resistance Epidemiology in Females with Cystitis (ARESC): implications for empiric therapy. Eur Urol 2008;54:1164–75.
6. Roland YA. Profil antibiotypique des bactéries responsables d'infection urinaire communautaire, thèse de doctorat en pharmacie, Bamako, Mali, 2006.

7. Sheerin NS. Urinary tract infection. Medicine 2015; http://dx.doi.org/10.1016/j.mpmed.2015.05.007

8. Kader A, Kumar A. Prevalence and antimicrobial susceptibility of extendedspectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in a general hospital. Ann Saudi Med 2005;25(3):239–42.

9. Paterson L, Bonomo A. Extendedspectrum beta-lactamases: a clinical update. Clin Microbiol Rev 2005;18:657–86.

10. Spanu T, Luzzaro F, Perilli M, Amicosante G, Toniolo A, Fadda G. Occurrence of extended-spectrum β-lactamases in members of the family Enterobacteriaceae in Italy: Implications for resistance to β-lactams and other antimicrobial drugs. Antimicrob Agents Chemother 2002;46(1):196–202.

11. Simon F, Kraemer P, De Pina J, Demortière E, Rapp C. Le risque nosocomial en Afrique intertropicale. Partie 2: les infections des patients. Med Trop 2007;67:197–203.

12. Sina H, Baba-Moussa F, Ahoyo TA, Mousse W, Anagonou S, Gbenou JD, et al. Antibiotic susceptibility and toxins production of staphylococcus aureus isolated from clinical samples from Benin. Afr J Microbiol Res 2011;5:2797–808.

13. Rotimi VO, Jamal W, Pal T, Sovenned A, Albert MJ. Emergenceof CTX-M-15 type extended-spectrum beta-lactamase-producing *Salmonella spp*. in Kuwait and the United Arab Emirates. J Med Microbiol 2008;57:881–6.

14. Drawz SM, Bonomo RA. Three decades of beta-lactamase inhibitors. Clin Microbiol Rev 2010;23:160–201.

15. Livermore DM. Bacterial resistance: origins, epidemiology, and impact. Clin Infect Dis 2003;36(Suppl 1):S11–23

16. Poirel L, Kampfer P, Nordmann P. Chromosome-encoded Ambler class A β-lactamase of Kluyvera georgiana, a probable progenitor of a subgroup of CTX-M extended-spectrum betalactamases. Antimicrob Agents Chemother 2002;46:4038–40.

17. Olson AB, Silverman M, Boyd DA, McGeer A, Willey BM, Pong-Porter V. Identification of a progenitor of the CTX-M-9 group of extended-spectrum beta-lactamases from *Kluyvera georgiana* isolated in Guyana. Antimicrob Agents Chemother; 2005;49: 2112–5.

18. Barguigua A, El Otmani F, Talmi M, Bourjilat F, Haouzane F, ZeroualiK. Characterization of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from community in Morocco. J Med Microbiol 2011;60:1344–52.

19. Tollentino FM, Polotto M, Nogueira ML, Lincopan N, Neves P, Mamizuka EM. High prevalence of bla(CTX-M) extended spectrum beta-lactamase genes in *Klebsiella pneumoniae* isolates from a tertiary care hospital: first report of bla(SHV-12), bla(SHV-31), bla(SHV-38), and bla(CTX-M-15) in Brazil. Microb Drug Resist 2011;17:7–16.

20. Elhani D, Bakir L, Aouni M. Changement de l'épidémiologie de Klebsiella pneumoniae productrice de -lactamases à spectre élargi. Ann Biol Clin (Paris) 2011;69:523–9.

21. Reinert R, Low DE, Rossi F, Zhang X, Wattal C, Dowzicky MJ. Antimicrobial susceptibility among organisms from the Asia/Pacific Rim, Europe and Latin and North America collected as part of TEST and the in vitro activity of tigecycline. J Antimicrob Chemother 2007;60:1018–29.

22. Bouchillon SK, Johnson BM, Hoban DJ, Johnson JL, Dowzicky MJ, Wu DH. Determining incidence of extended spectrum beta-lactamase producing Enterobacteriaceae, vancomycin-resistant *Enterococcus faecium* and Methicillin-resistant *Staphylococcus aureus* in 38 centres from 17 countries: the PEARLS study 2001–2002. Int J Antimicrob Agents 2004;24:119–24.

23. Cantón R, Coque TM. The CTX-M β-lactamase pandemic. Curr Opin Microbiol 2006;9:466–75

24. Eisler P. Deadly superbugs invade U.S. health care facilities. USA Today 2012. Available via https://www.usatoday.com/story/news/nation/2012/11/29/bacteria-deadly-hospital-infection/1727667/ (Accessed 1 December 2012).

25. CDC. Action needed now to halt spread of deadly bacteria: data show more inpatients suffering infections from bacteria resistant to all or nearly all antibiotics. The Centers for Disease Control, 2013. Available via https://www.cdc.gov/media/releases/2013/p0305_deadly_bacteria.html (Press release) (Accessed 5 March 2013).

26. Hawkey PM, Livermore DM. Carbapénème pour les infections graves. Br Med J 2012;344:e3236.

27. Patel G, Bonomo RA. 2013. Eaux orageuses à venir: émergence mondiale de carbapénémase. De face Microbiol 2013;4:48.

28. Elhani D. Les bêta-lactamases à spectre étendu: le défi s'accentue. Ann Biol Clin 2012;70(2):117–40; doi:10.1684/abc.2012.0686

29. Drieux L, Brossier F, Sougakoff W, Jarlier V. Phenotypic detectionof extended-spectrum beta-lactamase production in *Enterobacteriaceae*: review and bench guide. Clin Microbiol Infect 2008;14:90–103.

30. Philippon A, Arlet G. Lactamases de bacilles à Gram négatif: lemouvement perpétuel! Ann Biol Clin (Paris) 2006;64:37–51.

31. Pitout JD, Laupland KB. Extended-spectrum beta-lactamaseproducing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis 2008;8:159–66.

32. Thomson KS. Extended-spectrum-beta-lactamase, AmpC, and carbapenemase issues. J Clin Microbiol 2010;48:1019–25.

33. Livermore DM, Warner M, Mushtaq S. Evaluation of the chromogenic Cica-beta-Test for detecting extended-spectrum, AmpC and metallo-betalactamases. J Antimicrob Chemother 2007;60:1375–9.

34. Zhanel GG, Wiebe R, Dilay L, Thomson K, Rubinstein E, Hoban DJ, et al. Examen comparatif des carbapénèmes. Drogues 2007;67:1027–52.

35. Srinivasan A, Patel JB. Klebsiella pneumoniae organismes producteurs carbapenemase: Une once de prévention vaut vraiment la peine une livre de guérison. Infecter Contrôle Hosp Epidemiol 2008;29:1107–9.

36. Queenan AM, Bush K. Carbapénèmases: polyvalent β-lactamases. Clin Microbiol 2007;20:440–58.

37. Jamal W, Rotimi VO, Albert M, Khodakhast F, Nordmann P, Poirel L. Forte prévalence de VIM-4 et NDM-1 métallo- β-lactamase parmi résistant carbapénèmes Entérobactéries. J. Med. Microbiol. 2013;62:1239-1244.

38. Khan, l'Union africaine; Nordmann, P. 2012. Propagation des carbapenemase producteurs NDM-1: La situation en Inde et ce qui peut être proposé. Scand J Infect Dis 2012;44:531–5.

39. Donkor ES, Nortey T, Opintan JA, Dayie N, Akyeh ML. Sensibilité aux antimicrobiens de Salmonella typhi et Staphylococcus aureus et l'effet de certains médias sur les résultats des tests de sensibilité. Int J Microbiol 2007;4:2.

40. Varadi L., Luo JL., Hibbs DE., Perry JD., Anderson RJ., Orenga S. Les eaux souterraines, PW Méthodes de cation détection et identifié des bactéries pathogènes: passé, présent et futur. Chem Soc 2017;46:4818–32.

41. Wang L, Gu H, Lu X. Un faible coût rapide PCR en temps réel pour la détection de Klebsiella pneumoniae carbapénèmase gènes. Ann Clin Microbiol Antimicrob 2012;11:9.

42. Kelly AM, Mathema B, Larson EL. Résistantes aux carbapénèmes entérobactéries dans la communauté: une portée la revue. Int J Antimicrob 2017;50:127–34.

43. Woodford N, Turton JF, Livermore DM. Multirésistantes bactéries Gram-négatives: le rôle des clones à haut risque dans la diffusion de la résistance aux antibiotiques. FEMS Microbiol 2011;35:736–55.