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**African Journal of Microbiology Research**

*Review*

# Antibiotic resistance in Saudi Arabia and some Middle Eastern countries: Current status

## Shadi Ahmed Zakai

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Antibiotic resistance has become a major threat to human health worldwide, especially for serious infections, leaving limited choices for antimicrobials to be used. The World Health Organization (WHO) launched several strategies to control the spread of this resistance. However, in the Middle East the problem can become worse if these strategies are not applied due to the geographical location between developed and developing worlds, and the heavy misuse of antibiotics in the region. The current picture of antibiotic resistance in Saudi Arabia and some of the Middle Eastern countries represents high resistance rates among other regions to penicillin, erythromycin, fluoroquinolone, carbapenem and anti-tuberculosis drugs. Misuse of antibiotics is one of the major factors that contribute to the increase in antimicrobial resistance (AMR). This includes self-medication, incomplete dosage of the medication, missing doses, and re-use of excess antibiotics from previous prescription. Moreover, lack of adherence to infection control practice by healthcare professionals can contribute in spreading antibiotic resistance. An action plan has already been set by the WHO to limit antibiotic resistance. Application of this plan requires efforts of stakeholders from all regional countries to successfully stop the spread of AMR in the Middle East. Actions should include spreading public awareness of antibiotic resistance, as well as educational programs for healthcare professionals on infection prevention and control. National surveillances of antibiotic resistant bacteria that cause nosocomial infections are essential to identify outbreaks. Strict regulations regarding dispensing antibiotics should be imposed. Each country should take action to limit the spread of antibiotic resistance. If resistance is left uncontrolled, the region may face a time in which treatments of minor infections may become impossible.

**Key words:** Antimicrobial resistance, Saudi Arabia, Middle East, antibiotic misuse, penicillin, erythromycin, fluoroquinolone, carbapenem.

## INTRODUCTION

Antibiotics have been known to be the most effective method for the treatment of bacterial infections for decades. The discovery of penicillin in early 19th century has contributed in saving millions of lives by its ability to treat infectious diseases that were considered as the main killers in the pre-antibiotics ages. However, the

rapidly increasing development of multi-drug resistant (MDR) bacteria to a large number of antibiotics is now becoming a global concern, causing increased morbidity and mortality worldwide, an increase in health care bills, failure of treatments, and increased hospitalization and visits to out-patient clinics (Abobotain et al., 2013; Ricciardi et al., 2016). The World Health Organization (WHO) refers to the fast development of MDR bacteria as one of the major threats to public health internationally, as it severely limits the option of treating infectious diseases (Brookes-Howell et al., 2012; World Health Organization, 2014).

For this reason, the WHO sets many strategies to limit antibiotic resistance (World Health Organization, 2015). The key strategies are to improve public awareness and understanding of the correct use of antibiotics, to support the knowledge and evidence base, to minimize infection rates, to control antibiotic use in human and animals, and to develop the economic case for sustainable investment (World Health Organization, 2015).

Misuse of antibiotics is one of the main factors for the increase of antibiotic resistance worldwide. The concept of misuse involves self-medication, incomplete dosage of the medication, missing doses, taking insufficient doses, and re-use of left-over antibiotics from previous prescription (Abobotain et al., 2013).

Kardas et al. (2005) carried out a large systematic review to evaluate the frequency of compliance with treatment and the re-use of leftover antibiotics, as two main factors of antibiotic misuse in the community covering all continents with emphasis on North America and Europe. They reported that the mean compliance with antibiotics was 62.2% and mean use of leftover antibiotics was 28.6%.

According to the WHO, self-medication means selecting and using medicines by people to treat self-identified diseases or symptoms' (Aljadhey et al., 2015; World Health Organization, 1998). Over the Counter (OTC) medication is the drugs that can be bought, safely and effectively being used by the general public without requiring medical consultation by a healthcare professional, as defined by the US Food and Drug Authority (US Food and Drug Authority, 2015). Responsible self-medication is consumption of OTC medications, and it is an internationally appropriate practice, whereas buying prescription-medications without prescription from a healthcare professional is unsafe self-medication (World Health Organization, 1998). The OTC medication use can be valuable to reduce the frequency and the cost of visits to clinics. However, even misuse of OTC medications can be accompanied by antagonistic reactions, drug-drug interactions, overdosing, and other medication-related problems (Austin et al., 1999). Therefore, it is recommended to increase public awareness and education on the use of OTC medications, as well as to control dispensing OTC medication, in order to facilitate responsible self-care.

## The current status of antibiotic resistance in Saudi Arabia and some of the Middle Eastern countries

There is a remarkable increase in antibiotic resistance rates in common human pathogens in the Middle Eastern countries (Al-Tawfiq et al., 2009, 2010; Strahilevitz et al., 2007). Increased antimicrobial resistance was reported in several surveys in the last decade.

## Penicillin and other β-lactams

The rate of penicillin resistance in the Arabian Peninsula and Egypt was investigated by a literature search by Shibl et al. (2009) covering 1990 to 2007. They found a range of resistance to penicillin from 0 to 78%. The same study also reported an increasing range of penicillin resistance over many decades from 0 to 20% between 1980s and 1990s; it reached 40% in early 2000s, 50–80% between 2003 and 2005 (Shibl et al., 2009). Increasing resistant *S. aureus* strains are becoming a big concern, especially those acquired from hospitals. The rate of both hospital-acquired (HA) methicillin-resistant *S. aureus* (MRSA) infection and colonization has increased from 2.17 cases in every 1000 admissions to 3.90 cases in every 1000 admission only between 2000 and 2004 in Saudi Arabia (Balkhy et al., 2007). In another study, the rate of community-acquired (CA) MRSA ranged from 10 to 30 per 100,000 patient-days (Al-Tawfiq, 2006b). Furthermore, MRSA rates reached 32.0% of all *S. aureus* isolates from 13 Kuwaiti hospitals (Udo et al., 2008). A national surveillance on Gram-positive cocci carried out in Saudi Arabia showed that MRSA counted 32% of *S. aureus*, and penicillin G-resistant *Streptococcus pneumoniae* (*S. pneumoniae*) were 33%, and 26% were erythromycin resistant (Shibl et al., 2014). A study from Jeddah showed that nasal colonization by *S. aureus* was 25.3% of the 150 tested participants who were all senior medical students and interns, of which 6.7% were colonized by MRSA (Zakai, 2015). Similar study done in Riyadh, showed that 40% of healthcare workers were colonized by *S. aureus,* of which 18 were MRSA carriers (Al-Humaidan et al., 2015). Another study in Jeddah showed remarkably higher rate of MRSA nasal colonization of healthcare workers (Iyer et al., 2014). They reported an incidence rate of 76% of MRSA nasal colonization among the tested group of healthcare workers.

## Fluoroquinolones

The emergence of fluoroquinolone resistance in many regions in the Middle East has become a notable problem. In Saudi Arabia, from 2000 to 2006, resistance to ciprofloxacin by *Enterobacter cloacae* raised from 8.3 to 17.4% (Al-Tawfiq et al., 2009). Additionally, another

study in the same country showed a significantly increased resistant hospital-acquired isolates of *Klebsiella pneumonia* to ciprofloxacin. The resistance rate jumped from 2.6 to 23% in only six years (1998-2003) (Al-Tawfiq and Antony, 2007). Several other studies in the region were performed to examine the resistance rate to ciprofloxacin. In Oman, they reported ciprofloxacin resistance rate of 31% in urinary tract for *E. coli* isolates (Al-Lawati et al., 2000). In Lebanon, the resistance rate to ciprofloxacin in community isolates reached 25%, while it increased to 40–46% for hospital isolates (Fadel et al., 2004; Tohme et al., 2001). In Saudi Arabia, *E. coli* isolates were shown to be resistant to ciprofloxacin in 19% in outpatient urinary tract isolates, and 49% in inpatient urinary tract isolates (Al-Tawfiq, 2006a). Similar rates were found in Turkey, where ciprofloxacin resistance was reported in 42.1% of outpatients to *E. coli* (Yilmaz et al., 2009). Non-enteric species showed much higher rates of resistance. For instance, in the United Arab Emirates, resistance rate to ciprofloxacin was reported in 97.4% of *Pseudomonas aeruginosa* isolates (Al-Dhaheri et al., 2009).

## Carbapenem

In Saudi Arabia, carbapenem-resistance was remarkably noted in Gram-negative bacilli (GNB). This resistance was reviewed in the last ten years and compared to those of the 1990s (Zowawi et al., 2013). The same review also reported an increased prevalence of extended spectrum beta-lactamase (ESBL) producing isolates in Saudi Arabia. Some institutes reported an ESBL rate of 29% among *Escherichia coli* (*E. coli*) and 65% among *K. pneumoniae* (Zowawi et al., 2013).

Resistance of *Acinetobacter baumannii (A. baumannii)* to carbapenem (CRAB) showed a dramatic increase in Saudi Arabia. A study from Riyadh (Al-Obeid et al., 2015) demonstrated a decrease in susceptibilities of *A. baumannii* to meropenem and imipenem from 64-81.2 to 8.3-11%, in only six years between 2006 to 2012. Additionally, a study from the same region showed that multidrug resistance was reported in 14–35.8% of *Acinetobacter spp.* isolates (Al-Tawfiq and Mohandhas, 2007). In 2002, an outbreak of MDR *A. baumannii* was reported in one of the intensive care units (ICUs) in Qatar (El Shafie et al., 2004). A total number of 21 patients were included in this outbreak, all their infection/colonization were hospital-acquired. In the same study, a similar strain was isolated from the hospital equipment, environment, and also from hands of the healthcare workers. Another study reported similar outbreak of an epidemic strain of *A. baumannii* in 87% of patients undergoing tracheostomy at a tertiary care hospital in Riyadh, Saudi Arabia (Mah et al., 2001).

Colistin is considered as the final antibiotic of choice to be used against many of carbapenem-resistant Gram

Negative Bacilli (GNB). Nevertheless, resistance to colistin has already been reported (Zowawi, 2016). It has been known that resistance to colistin is chromosomally mediated, which means it is not transmissible between bacterial cells. In China, however, an emergence of plasmid-mediated colistin resistance (*mcr-1*) was reported in both human and animals (Liu et al., 2016). A study reported the presence of *mcr-1* gene in *E. coli* strains isolated from Saudi Arabia, Bahrain and United Arab Emirates, which suggests the possibility of spreading of *mcr-1* carrying GNB in more isolates and between other species in the Arabian Peninsula (Sonnevend et al., 2016).

## Anti-tuberculosis drugs

Anti-tuberculosis (anti-TB) drug resistance is becoming a critical problem for many countries, especially in those where the infection is endemic. A Saudi national survey on anti-TB drug resistance reported that the MDR TB phenotype was demonstrated in only 1.6% of total TB (Al-Hajoj et al., 2013). The WHO Global Project investigated the MDR-TB across 79 countries worldwide between 1999 and 2003. They found MDR-TB in 1.1% of new cases and 6.9% of retreatment patients (Aziz et al., 2006). In contrast, the regional surveillance estimated the rates of MDR-TB, which ranged from 2.3% in new cases to 52% in retreatment cases (Shamaei et al., 2009).

## Regional factors contributing to the increase in the emergence and spread of antibiotic resistance

In the Middle East, the scenario of antibiotic resistance is unique. The geographical location of the Middle East connects the developed and developing worlds, thus, it is exposed to a variety of resistant bacteria from both worlds. For instance, oxacillin-resistant *S. aureus,* penicillin-resistant *Streptococcus pneumoniae* and multi-drug resistant (MDR) *Mycobacterium tuberculosis* (Al-Tawfiq et al., 2010).

During Hajj pilgrimage, millions of Hajj pilgrims visit two major cities in the Kingdom of Saudi Arabia annually. The gathering of this large human population brings imported and endemic diseases to be well transmitted between pilgrims from different countries and local pilgrims and residents, and then exported across many countries later (Al-Tawfiq et al., 2010; Moore et al., 1988). International travel is an important risk factor for the acquisition and transmission of infectious diseases, including those caused by high diversity of antibiotic resistant bacteria. A study assessed the carriage status of diverse bacteria in Hajj pilgrims before and after they travelled to Hajj (Leangapichart et al., 2016). Interestingly, the diversity of bacteria isolated from samples that were collected after the return from Hajj was significantly higher than those

collected before, in particular, carbapenemase-producing *A. baumannii* and *E. coli*. The high acquisition rate of diverse bacteria in Hajj season may be referred to the overcrowded conditions of the pilgrims in small areas. An increase by 16.5% in septicaemia cases was reported in Makkah during Hajj season due to the arrival of external visitors (Zowawi et al., 2013). In a study executed to evaluate the prevalence of MRSA carriage among Hajj pilgrims, it was found that *S. aureus* was present in 20.6% of the tested samples (*n* = 411), of which 1.46% were MRSA (Memish et al., 2006). Another common infection among hospitalized Hajj pilgrims is tuberculosis. A study done during the Hajj season of 64 patients with pneumonia, it was found that *M. tuberculosis* was the most common causative bacterial agent of pneumonia (Alzeer et al., 1998).

Uncontrolled use of antibiotics is one of the major factors that contribute to the increase of antimicrobial resistance (AMR). One means of antibiotic misuse is the uncontrolled use by patients, such as self-medication, noncompliance with antimicrobial therapy, taking sub-optimal dose, and consumption of leftover medication. The availability of OTC antibiotics that can be obtained without prescription was a major concern in Saudi Arabia before the Saudi Ministry of health set strict regulations in early 2018 for antibiotic prescription by the Executive Regulations of Health Practice Law, which prohibit pharmacists from dispensing any drug without a prescription issued by a doctor with a license to practice in the Kingdom. Self-medication was reported in 11.6% in a sample of Saudi parents (60.5% mothers and 39.5% fathers). Half of them claimed that antibiotics could be used for their children in cases of runny nose, cough, sore throat or fever. However, more than half (57.7%) believe that they can reduce severity and shorten duration of illness, 68.6% believe they can relieve viral infection, and 28.7% claim they can be stopped upon improvement (Abobotain et al., 2013). Another study in the Eastern region of Saudi Arabia reported that 67% of the parents tried self-medication once or more, and 37.7% of them used leftovers antibiotics (Al-Shawi et al., 2018). Misuse of antibiotics by patients is known to be common in the region. A Jordanian study reported self-medication rates of 23.1-39.5% (Al-Azzam et al., 2007; Al-Bakri et al., 2005). Higher rates (53%) were reported in Iran (Sarahroodi and Arzi, 2009). Another means of antibiotic misuse is the uncontrolled self-prescription by pharmacists. A Saudi study showed that 82% of pharmacists recommended self-prescription to treat symptoms of urinary tract infections, in which fluoroquinolone is the ordinarily dispensed antibiotic (Al-Ghamdi, 2001). A study in the Eastern region of Saudi Arabia revealed that only one out of 88 pharmacists refuse to sell antibiotics without prescription, while in Riyadh, 77.6% of pharmacists sell antibiotics without prescription (Zowawi et al., 2013). Physicians also have a role in antibiotic misuse in the area. An Iranian study showed that 37-40% of physicians prescribed antibiotics for cases that did not require antibiotic treatments. The Iranian physicians refer to patient pressure to prescribe more medication, particularly antibiotics (Amidi et al., 1975).

Another factor that contributes to the increase of antibiotic resistance is the lack of compliance of patients to the prescribed antimicrobial therapy. Comparing the status in the Middle East to elsewhere, data have shown better compliance in this region than somewhere else. A meta-analysis study revealed a rate of 72.4% of compliance with prescribed antibiotics, whereas internationally it was 58–68% (Kardas et al., 2005). Unfortunately, this is not the case with anti-TB agents. Data from Saudi Arabia have shown mixed results regarding compliance with anti-TB agents. In one study of 628 patients, noncompliance rate with the prescribed regimen was 43.8% (Al-Hajjaj and Al-Khatim, 2000). Another study found distinct gender variations showed a low noncompliance rate among females (15.3%), while a much higher rate among males (44%) (Samman et al., 2003).

Adherence to infection control practice by healthcare professionals is an essential factor in limiting the spread of antibiotic resistance. Hand hygiene of healthcare professionals was assessed in Makkah during the Hajj season in 2011 (Bukhari et al., 2011). The overall rate of compliance with hand hygiene was 50.3%, with highest rate among nurses (52.2%), followed by doctors (49.1%), and technicians (42.8%).

## Antibiotic prescription and respiratory tract infections

Physicians prescribe antibiotics for upper respiratory tract infection (URTI) in children (Sa'ed et al., 2015). Nevertheless, URTI is most commonly caused by viruses (Harnden et al., 2007). Moreover, some bacterial infections causing URTI are self-limiting and do not require antimicrobial treatments, such as otitis media and sinusitis (Panagakou et al., 2011). Antibiotics are frequently misused by parents to treat upper respiratory tract infections, which are mainly caused by viruses. Parents believe they can reduce the severity of illness symptoms, although antibiotics have no effect on reducing symptoms nor shorten the time of recovery (Friedman et al., 2011). A Palestinian study addressed the parents' knowledge, attitude and practices on antibiotic use for children suffering from URTIs. Nearly 73% of parents choose antibiotics as a treatment for URTIs, 68% for earache, and 64% for fever (Sa'ed et al., 2015). A study done in a Jordanian community focused on the consumption of antibiotics, found that 46% of antimicrobial medications were dispensed without a prescription, either through self-medication (23.2%) or pharmacist recommendation (23.1%) (Al-Bakri et al.,

2005). Of dispensed antibiotics in this outpatient setting, almost one-third (29%) was inappropriate for prescribed and 34% for non-prescribed antibiotics. Most of these patients used these antibiotics to treat upper respiratory tract infections (URIs) symptoms. A study in Riyadh done on community pharmacies found that 49% of purchased medications were dispensed without prescription, in which antibiotics account for 22% of prescription medications that were dispensed without prescription. The commonest reasons for buying such medication without having a proper prescription from a physician were that symptoms were very minor to seek medical advice (54%), time saving (40%), and former consumer knowledge about the symptoms (40%) (Aljadhey et al., 2015). Another study carried out on an outpatient population in Alexandria found that 55% of oral antibiotics were inappropriately used (Koura et al., 1999). Additionally, in Kuwait a study examined antibiotic dispensing practices for children attending a primary healthcare center. They reported excessive prescription of antibiotics for 39% of the children, of whom 72% had respiratory tract infections (Najdi et al., 1988). Greater rates of inappropriate antibiotic prescribing were reported in Saudi Arabia. A study performed at a primary healthcare center in northern region of Saudi Arabia reported that antibiotics were prescribed to 87.8% of patients with upper respiratory infection (URI) (El-Gilany, 2000). In another study, antibiotics were prescribed to 51.6% of patients with URI symptoms in Bahrain (Senok et al., 2009).

## Action plan is needed to limit the antibiotic resistance in the region

Antibiotic resistance shows a serious burden to the public health in the Middle East. For this, the WHO started the Global Action Plan on Antimicrobial Resistance (World Health Organization, 2015). This plan was committed by most of the region countries, including Saudi Arabia in the World Health Assembly in 2015. The plan addressed five main goals; to increase awareness, to strengthen knowledge through surveillance and research, to decrease the rate of infection, to control the use of antimicrobial agents, and to develop economic tools for sustainable investment to support the need in all countries in regards to new medicines, diagnostic tools, vaccines, and other interventions. To realize this action plan, some regional countries launched their own national AMR action plans, and their main common goal is to challenge the AMR in both human and animal sectors.

Spreading community awareness to AMR through different channels is necessary to tackle the AMR in the Middle East, particularly in Saudi Arabia as it is visited by large crowds during Hajj seasons with visitors from all over the world. Nationwide awareness campaigns should be initiated through different channels to ensure as wide

delivery of the message as possible. Nowadays, this can be easily done via TV, radio, and social media channels (Zowawi et al., 2015). Governmental efforts can help to increase public awareness regarding antibiotic misuse. A good example is the French national 5-year program that reduced the antibiotic prescription by 26% between 2002-2007 (Sabuncu et al., 2009).

Educational program for healthcare professionals on infection prevention and control is crucial to limit the spread of AMR. Education on hand hygiene compliance can play an important role in limiting the spread of outbreaks in hospitals and community.

Due to the global increased prevalence of AMR bacteria, and the necessity for synchronized efforts for interventions, there should be matching between trends of resistance and amount of antibiotic consumption by local records to define the problem and allow appropriate interventions. Randomized regional studies can aid minimizing antibiotic resistance; however, expanded work is required in the Middle East region. Establishing national surveillance of AMR bacteria that cause nosocomial infections can help to identify outbreaks.

Last but not least, to limit the misuse and self-medication of antibiotics in the region, there should be strict regulation regarding selling these agents. Therefore, accessibility in all Middle Eastern countries must be restricted to antibiotics prescribed by physicians.

## Conclusion

It is clear that AMR is a serious current global burden that threat public health, which requires focused attention, and the problem is also occurring in the Middle Eastern countries. If AMR is left uncontrolled, the region may face a 'post-antibiotic era', in which treatments of minor infections may become impossible, mortality rate may increase as well as the treatment costs and newer complex, more expensive antibiotics will be required. All countries should take action for limiting the spread of AMR. Prompt actions may embrace national surveillance of AMR at both hospital and community level. Educational programs of infection prevention and control should be initiated toward the same goal. Raising public awareness of AMR is also required to control misuse of antibiotics. Finally, the use of antibiotics should be controlled in hospital and community pharmacies.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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## African Journal of Microbiology Research

*Full Length Research Paper*

# First report of the types TEM, CTX-M, SHV and OXA-48 of beta-lactamases in *Escherichia coli*, from Brazzaville, Congo

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The objective of this study was to evaluate the emergence of beta-lactam resistance related to extended spectrum beta-lactamase (ESBL) encoding genes (TEM, SHV, CTX-M-1 and OXA-48) in *Escherichia coli* from Brazzaville. In the period between January 2016 and May 2017, 89 strains in *E. coli* were isolated from hospitalized patients, outpatients and domestic sewage. The *E. coli* strains were identified by the API 20E system. An antibiogram was performed on isolated trains by the disk diffusion method. The ESBL phenotype was detected using the synergistic technique according to CA-SFM (ESBL). Genes were detected using PCR and characterized by sequencing. An overall prevalence of 48.31 (43/89) and rates of 74.42, 23.26, 9.30, and 6.97% for blaTEM genes blaCTX-M-1, blaSHV and blaOXA-48 were detected. 25.58% were community strains and 74.42% hospital. The majority were isolated urine (81.40%) and the urology department was more represented. Except for imipenem, colistin strains of ESBL showed high resistance to other antibiotics than non-yielding ones (p <0.05). This high prevalence of detected ESBL, the high level of resistance to antibiotics raises fears of a high risk of multidrug-resistant bacteria and call on the authorities for a policy of monitoring resistance.

**Key words:** *Escherichia coli*, extended spectrum beta-lactamase (ESBL), carbapenemases, Brazzaville.

## INTRODUCTION

Infectious diseases remain a major public health problem, especially in Africa (Ouedraogo et al., 2017). Bacterial infections are the most dominant and are responsible for most of the nosocomial and community infections. Based on their wide spectrum, mechanism of action, low toxicity and low cost, antibiotics including the beta-lactam family such as penicillins, cephalosporins and carbapenems are the most commonly used for treating infection diseases

(Livermore, 1995). Over the past year, a significant increase in antibiotic resistance to Enterobacteriaceae has been documented as well. According to the WHO (2014) report on global surveillance of antimicrobial resistance, antibiotic resistance is a serious public health problem. In competitive environments, microorganisms including *Streptomyces*, *Nocardia*, *Actinomadura*, and *Penicillium* species, can produce natural molecules allowing the inhibition of other competitors (Baquero and Coque, 2013). To avoid self-toxicity, these microorganisms have intrinsically developed antibiotic resistance. Enterobacteriaceae are classified in different groups according to antibiotic resistance (Philippon et al., 1989). Indeed, the abuse of antimicrobials led to the selection of multi-resistant strains, particularly those resistant to beta-lactams isolated from urinary infections, pulmonary infections, and septicemia, with an increasing frequency in hospitals (Lucet et al., 1996). In Enterobacteriaceae, different enzymes are produced depending on their membership in the different groups. The most commonly cited are chromosomal penicillinase, chromosomal cephalosporinase, chromosomal cefuroximase and chromosomal extended spectrum beta-lactamase (ESBL). *Escherichia coli* is a dominant Enterobacteriacea found in the human commensal flora, especially in the digestive tract (Ahoyo et al., 2007).

ESBLs are serine-type inactivation enzymes. With the exception of OXA SSBLs (class D), ESBLs are class A beta-lactamases according to the Ambler classification. More than 300 ESBLs have been described to date (Elhani, 2012). They are characterized by a great diversity. The majority of ESBLs are derived from TEM and VHS enzymes but new ESBLs have been described such as CTX-M (for cefotaximase), OXA (for oxacillinase), PER (for *Pseudomonas aeruginosa*), VEB (for Vietnam ESBL), GES (for Guyana extended-spectrum beta-lactamase), TLA (for TEM Like activity), BES (for Brazilian extended spectrum beta-lactamases), SFO (for *Serratia fonticola*) and FEC (fecal *E. coli*) (Elhani, 2012).

ESBLs constitute a large family of bacterial enzymes belonging to classes A, C and D based on the Ambler classification (Hall and Barlow, 2018), and capable of hydrolyzing penicillins, cephalosporins and aztreonam. They do not hydrolyze carbapenems or cephamides. This capacity to hydrolyse antibiotics has also been demonstrated in *E. coli* and *Klebsiella* species strains. Since the discovery of ESBL-producing bacteria in 1990, most of those detected are the conventional TEM and SHV types, which spread predominantly in hospital settings, including *Klebsiella pneumoniae* and *Enterobacter* species; they are associated with nosocomial outbreaks in intensive care units (Hall and Barlow, 2018). Recently, a new blaCTX-M gene encodes CTX-M enzyme has been detected. Community strains of *E. coli*, mainly responsible for urinary tract infections, could express blaCTX-M genes encoding CTX-M enzyme (Elhani, 2012)

The *blaTEM*, *blaSHV*, *blaCTX-M* and *blaOXA* genes have been described in several epidemiological studies in Europe, Asia, the USA and South America (Bush and Jacoby, 2010; Winokur et al., 2001; Villegas et al., 2008).

Organisms expressing these genes are widespread throughout the world but some geographical regions have a significantly higher prevalence rate such as South America, Asia and Europe. These prevalence rates have been evaluated by large microbial resistance surveillance programs such as SENTRY and MYSTIC and there is a steady increase in there prevalence (Goossens, 2005; Yano et al., 2013).

In Africa, prevalence has recently been estimated at less than 15% (Tansarli et al., 2014). Several previous studies reported the presence of ESBL at rates of 1.3% in Morocco (Bourjilat et al., 2011); 3.8% in Senegal (Sire et al., 2007); 4% in Central African Republic (Lovollay et al., 2006), 12 and 16% in two studies in Cameroon (Gangoue et al., 2005; Lonchel et al., 2012); 22% in Benin (Ahoyo et al., 2007).

Classes A and D enzymes are commonly found in Africa with a predominance of *bla*CTX-M-15 (Storberg, 2014). In Congo-Brazzaville, a prevalence of 73.8% of *E. coli* strains harboring beta-lactamase phenotype was reported (Moyen et al., 2014). To our knowledge, no study has been performed on the characterization of Enterobacteriaceae resistance genes.

Based on the aforementioned, this study was carried out to evaluate the emergence of beta-lactam resistance related to genes encoding ESBL (TEM, SHV, CTX-M-1 and *OXA-48*) in *E. coli* from Brazzaville.

## MATERIALS AND METHODS

### Bacterial strains

From January 2016 to May 2017, *E. coli* isolates were isolated from various pathological samples (urine, vaginal swabs excretions, sperm, blood and pus), environmental samples (domestic sewage).

Collected samples were inoculated on Mac Conkey agar or methylene blue eosin (EMB) agar and incubated for 24 h at 37°C in the Bacteriology-Virology Laboratory of the CHU in Brazzaville and the Exau-Kenn Laboratory.

The suspected *E. coli* isolates were purified and biochemically identified using API 20E® galleries (bioMerieux, France) and the strains were stored in Luria-Bertani (LB) liquid medium (Sigma-Aldrich, France) supplemented with 10% glycerol at -80°C for the duration of the study.

### Study of the sensitivity

The antibiograms were made by diffusion in agar medium on Mueller-Hinton medium (Becton Dickinson, Le Pont de Claix, France) according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM) (Bonnet et al., 2010). The agar plates were inoculated with a swab from a culture inoculum calibrated at 0.5 McFarland before the deposit of the disks impregnated with antibiotic (Bio-Rad, Marnes-

**Table 1.** Primers used for conventional PCR and sequencing.

| Gene | Primer | Sequence (5' → 3') | Accession number | Product size (bp) |
|---|---|---|---|---|
| *bla*TEM | F<br>R | ATGAGTATTCAACATTTCCGTG<br>TTACCAATGCTTAATCAGTGAG | KJ939560.1 | 861 |
| *bla*SHV | F<br>R | TTTATGGCGTTACCTTTGACC<br>TCCCATGATGAGCACCTTTAAA | AF124984.1 | 1051 |
| *bla*CTX-M1 | F<br>R | CAGCGCTTTTGCCGTCTAAG<br>TTTGCGATGTGCAGTACCAGTAA | JQ397665.1 | 944 |
| *bla* OXA-48 | F<br>R | TTGGTGGCATCGATTATCGG<br>GAGCACTTCTTTTGTGATGGC | AY236073 | 744 |

bp: Base pair; F: forward ; R: reverse.

La-Coquette, France). After incubation of agar plates at 37°C for 18 to 24 h inhibition diameters, due to antibiotic concentration gradients established from the disc, they were compared to the critical values given by CA-SFM, thus allowing definition of the susceptibility or resistance of a strain to an antibiotic.

ESBL production was confirmed phenotypically according to CA-SFM recommendations, including the MH agar synergy test between clavulanic acid disc and cephalosporin discs of the 3rd (ceftriaxone: CRO, cefipime: FEP). The carbapenemase OXA-48 phenotype was determined when there was a decrease in inhibition diameter around the Ertapenem disk on MH agar in an imipenem-susceptible strain (Yagoubat et al., 2017). Sixteen (16) antibiotics were tested: amoxicillin AMX (25 µg), amoxicillin/clavulanic acid AMC (20/10 µg), cefipime FEP (30 µg), Piperacillin-Tozobactam TZP (75/10 µg), cefalotin CF (30 µg), ceftriaxone CRO (30 µg) ERT ertapenem (10 µg), imipenem IMP (10 µg), fosfomycin FF (50 µg), nitrofuran FNitro (300 µg), trimithoprim + sulfaphametxazole SXT (1.25/23.75 µg), amykacin (30 µg), ciprofloxacin CIP (5 µg), doxicycline DO (30UI), CT colistin (50 µg) and gentamycin GEN (15 µg). The different strains tested were classified into Sensitive (S), Intermediate (I) and Resistant (R) categories. The strain of *E. coli* ATCC 25922 was used as a sensitive reference for susceptibility testing.

**DNA isolation**

Bacteria isolates were separately grown overnight on a MacConkey agar plates at 37°C. Subsequently, colonies were picked up using a sterile 10 µL plastic loop and transferred into 200 µL sterile water (Nouria et al., 2016). Total DNA was extracted with the NucliSENS easyMag automat (Biomérieux, France) according to the manufacturer's protocol. DNA was eluted in a final volume of 200 µL. The extracted DNA was stored at -70°C for further analysis.

**PCR Detection of ESBL and carbapenemase genes**

Molecular detection of genes was performed on all the strains by the conventional PCR technique using a T100 thermocycler (Bio-Rad, France) using the specific primer pairs for the *bla*TEM genes (Kruger et al., 2004), blaCTX-M1 (Roschanski et al., 2014), *bla*SHV (Yagi et al., 2000) and *bla*OXA-48 (Poirel et al., 2011) shown in Table 1.

The PCR was carried out in a final volume of 50 µL containing: 1 µL of each primer at 20 µM, 5 µL of 10X PCR reaction buffer, 1 µL of the dNTPs, 0.25 µL of Taq DNA polymerase (Eurogentec,

Belgium), 36.75 µL of distilled water sterile, and 5 µL of crude DNA extract. Standard PCR conditions at 95°C for 15 min; followed by 35 cycles of amplifications each comprising a hybridization step at 55°C for 50 s, an elongation step at 72°C for 1 min, and a final extension step at 72°C for 7 min.

The amplification products were detected by electrophoresis using 1.5% agarose gels containing BET ethidium bromide diluted to 125 µL per 50 ml agarose gel heated in an electrophoresis vat containing buffer TBE (TRIS, Borate, EDTA) at 0.5%. The migration is done for 25 min at a voltage of 135V (Invitrogen, Leek, The Netherlands), as well as a DNA molecular weight marker (BenchTop pGEM®DNAMarker, Promega, Madison, Wisconsin, USA). Visualization of the gels was performed using the leaning ADNPPEM marker (Promega, Madison, Wisconsin, USA) under ultraviolet illumination.

**DNA sequencing**

Classical PCR products positive for ESBL genes were purified using the NucleoFast 96 PCR plate (Machery-Nagel EURL, France) and sequenced using BigDye terminator chemistry on a automatic sequencer ABI3730 (Applied Biosystems, Foster City, Californie, États-Unis).

The sequences obtained were assembled and corrected with Codon Code Aligner software. Sequence alignment and analyses were performed using the basic local alignement search tool (BLAST) software available on the website of the National Information Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 7 (Kumar et al., 2017).

**Statistical analysis**

For the data analysis, intermediate category strains were counted among the resistant (I + R). The data were analyzed using GraphPad Prism 7 software. The Chi-square test ($\chi^2$) was used to compare the resistance frequencies between the different parameters studied. The difference between frequencies was considered significant when the *p*-value was less than 0.05.

**Ethical clearance**

The study was approved by the ethics committee of Marien

**Table 2.** Antibiotic susceptibility of ESBL-producing isolates (n=43) and no ESBL-producing isolates (n=46).

| Antibiotic | *E. coli* ESBL+ *(N=43)* % (n) R | *E. coli* ESBL- *(N=46)* % (n) R | *p*-value |
|---|---|---|---|
| AMX | 100 (43) | 100 (46) | - |
| AMC | 100 (43) | 93.48 (43) | 0.330 |
| TZP | 46.51 (20) | 0 | 0.0001** |
| CF | 95.6 (22) | 67.39 (31) | 0.119 |
| CRO | 79.07 (34) | 17.39 (8) | 0.0001** |
| FEP | 37.20 (16) | 0 | 0.00001** |
| ERT | 9.30 (3) | 0 | 0.0684 |
| IMP | 0 | 0 | - |
| CS | 0 | 0 | - |
| AK | 9.30 (4) | 0 | 0.0344** |
| GEN | 34.88 (15) | 0 | 0.00001** |
| CIP | 13.95 (6) | 0 | 0.0095** |
| FF | 46.51 (20) | 0 | 0.00001** |
| F | 69.77 (30) | 0 | 0.00001** |
| DO | 53.49 (23) | 97.82 (45) | 0.00001** |
| SXT | 93.02 (40) | 95.65 (44) | 0.590 |

AMX: Amoxicillin; AMC: amoxicillin/acid clavulanic; TZP: piperacillin-tozobactam; CF: cefalotin; CRO: ceftriaxon; FEP: cefipim; ERT: ertapenem; IMP: imipenem; CS: colistin; AK: amykacin; GEN: gentamycin; CIP: ciprofloxacin; FF: fosfomycin; F: nitrofurantoin; DO: doxycillin; SXT: trimethoprim+sulfaphametaxazol. N: strains number. R: resistance. (**): $\alpha$ < 0.05 (exact Fisher test, 2-tailed) indicates statistically significant differences in resistance rates between ESBL *E. coli* and no-ESBL *E. coli.*

Ngouabi University. The protocol was reviewed and accepted by the authorities of Brazzaville hospitals (this allowed us to conduct our study). During the study period, we ensured the confidentiality of the results and the anonimate of the patients.

## RESULTS

### Bacterial strains

Eighty-nine (89) isolates of *E. coli* non-redundant were collected from two types of samples: an environmental sample consisting of household wastewater collected in a few neighborhoods of Brazzaville and a clinical sample taken from samples for the diagnostic purpose of hospitalized patients and those seen externally at the Makelekele base hospital and at the University Hospital Center of Brazzaville.

These samples consisted of five (5) blood cultures, sixty four (64) urine, one (1) vaginal sample, one (1) spermoculture and four (4) pyocultures, distributed as follows (Table 4): 14 *E. coli* originated from household sewage and 75 from hospital, twenty 20 strains were from outpatients and fifty five (55) straines from hospital services including 3 surgical, 9 urology care, 5 pediatric care (infant and grandchild), 4 infectious deseases, 10 cardiology care, 9 metabolic deseases, 9 general medicine, 3 neonatal and 3 intensive care unit (Table 3).

The resistance profiles of 89 isolates of *E. coli* studied on the 16 antibiotics used show a 100% sensitivity for imipenem and colistin, in *E. coli* ESBL+ and *E. coli* ESBL- isolates.

Low resistance was observed for Ertapenem 9.30% (3/43). The frequencies of resistance to amoxicillin, amoxicillin/clavulanic acid and cephalothin, which are antibiotics used in the first-line treatment of urinary tract infections, are relatively high in the two groups, but moreso for the strains of ESBL+ than ESBL- (Table 2). This difference is not statistically significant (p > 0.05). Aside from amoxicillin, amoxicillin/clavulanic acid, cephalothin, ertapenem, imipenem, colistin, and trimethoprim + sulfaphametxazole, there is a statistically significant difference between *E. coli* ESBL+ and *E. coli* ESBL- (Table 2).

Regarding to aminoglycosides, there was fairly marked resistance for gentamicin in ESBL *E. coli* isolates only at 34.88%, but it was only 9.30% for amykacin. In addition, with respect to fluoroquinolones, a resistance level of 13.95% was detected for ciprofloxacin; Furans remain resistant to 69.77%; as for the sulfamides and tetracyclines, the latter remain resistant in the two groups of strains studied (Table 2).

**Table 3.** Prevalence of *E. coli* isolates ESBL-producing based on their origin (N=89).

| Origin | *E. coli* ESBL+ % (n) | *E. coli* ESBL- % (n) | Total |
|---|---|---|---|
| surgery | 100 (3) | 0 | 3 |
| Infectious diseases | 75 (3) | 25 (1) | 4 |
| Pediatrics | 100 (5) | 0 | 5 |
| Urology | 77.78 (7) | 22.22 (2) | 9 |
| Cardiology | 40 (4) | 60 (6) | 10 |
| Metabolic diseases | 55.56 (5) | 44.44 (4) | 9 |
| Intensive care | 33.33 (1) | 66.67 (2) | 3 |
| General medicine | 33.33 (3) | 66.67 (6) | 9 |
| neonatology | 33.33 (1) | 66.67 (2) | 3 |
| communal | 32.35 (11) | 67.65 (23) | 34 |
| Total | 48.31 (43) | 51.69 (46) | 89 |

$\chi^2$=18.11;  ddl=9;  $\alpha$ =0.0339.

**Table 4.** Frequency of *E. coli* (N=89) and nature of samples.

| Nature of samples | *E. coli* ESBL+ % (n) | *E. coli* ESBL- % (n) | p-value |
|---|---|---|---|
| Urine | 81.40 (35) | 63.04 (29) | |
| Sewage | 2.32 (1) | 30.23 (13) | |
| Blood | 6.97 (3) | 4.35 (2) | |
| Pus | 6.97 (3) | 2.17 (1) | 0.00001 |
| PV | 0 | 2.17 (1) | |
| Sperms | 2.32 (1) | 0 | |
| Total | 48.31 (43/89) | 51.69 (46/89) | |

$\chi^2$=26.19; ddl=5;  $\alpha$=0.00001. Pv: Vaginal collection ; ESBL: extended spectrum beta-lactamase.

## Characterization of ESBL genes

Figure 1 shows the *bla*TEM, *bla*SHV, *bla*CTX-M-1 and *bla*OXA-48 genes with bands of respective sizes of 861, 1051, 944 and 744 base pairs.

Figure 2 shows the evolutionary history of ESBL genes (*bla*TEM, *bla*SHV, *bla*CTX-M-1). The optimal tree with the sum of branch length = 3.01339548 is shown. Evolutionary distances were calculated using the composite maximum likelihood method and are expressed in units of the number of base substitutions per site. All positions with gaps and missing data have been eliminated. There was a total of 479 positions in the final dataset.

The prevalence of ESBL genes on 89 isolates of *E. coli* was 48.31% (43/89). 25.58% (11/43) were community strains and 74.42% (32/43) were hospital strains. With a statistically significant difference in the production of genes that code for ESBLs between community strains and hospital strains, *p*-value < 0.05 ($p$ = 0.0178).

The *bla*TEM gene was the most common with 74.42% (32/43), followed by *bla*CTX-M1 23.26% (12/43) and

*bla*SHV 9.30% (4/43) and then *bla*OXA-48 6.97% (3/43) (Table 5). The following set: a triple expression *bla*TEM-SHV-OXA-48 was identified in a strain (2.33%) isolated from urine in a patient hospitalized in general practice, followed by a double expression *bla*TEM-CTX-M1 respectively in six isolates (13.95%). The *bla*TEM gene alone was present in twenty five (25) strains (58.14%), blaCTX-M1 in six (6) isolates (13.95%) and *bla*SHV in thee (3) isolates (6.97%) and then *bla*OXA-48 in two isolates (4, 65%) (Table 5).

The distribution of strains according to the hospital service shows a predominance of ESBL producing strains for the surgical and pediatric departments at 100%. The urology department comes in second place with 77.78%; followed by infectious diseases 75%, metabolic diseases (55.56%) and cardiology (40%). General medicine, pediatric intensive care and neonatology departements come last (33.33%) (Table 3).

Sequencing of PCR amplification products and after comparison based on NCBI data revealed that: all 32 *bla*TEM-positive strains were all TEM-1 AAR25033.1 (Table 5); the 12 CTX-M strains of group 1 were all CTX-
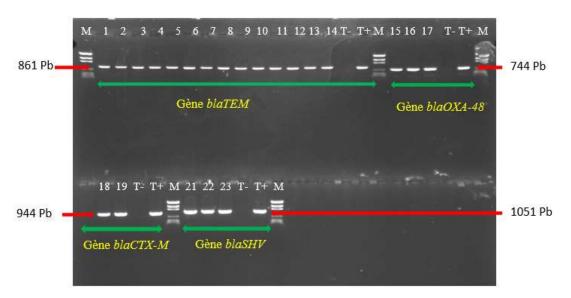
**Figure 1.** 1.5% agarose gel electrophoresis showing simplex PCR amplification products for detection of the genes ESBL. Lane T: Negative control; Lane M: molecular weight marker (Invitrogen, 100 bp DNA Ladder); Lane T+: Positive Control (blaTEM, blaCTX-M-1, blaSHV and blaOXA-48); Lane 1-14: positive samples for *bla*TEM (861 bp); Lane15-17: positive samples for *bla*OXA-48 (744 bp); Lane 18-19: positive samples for *bla*CTX-M-1 (944 bp); Lane 21-23: positive samples for *bla*SHV (1051 bp).
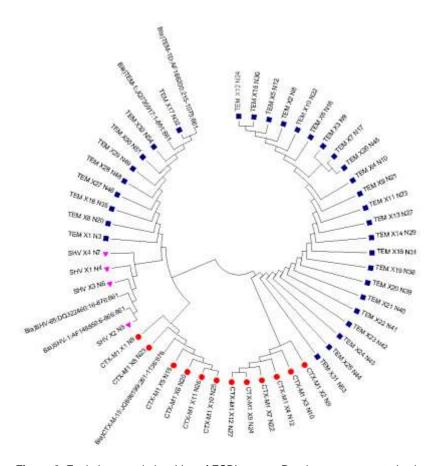


**Figure 2.** Evolutionary relationships of ESBL genes. Dendrogram generated using BioEdit and MEGA7.1 from the blaTEM gene set (blue square), *bla*CTX-M-1 (Red dot) and *bla*SHV (Pink Triangle) by comparing their relationship to each other and to other genes ESBL deposited at GenBank (black text). The lengths of branches are indicative of kinship.

**Table 5.** Characterization of the ESBL genes of the 43 *E. coli* isolates of hospital and community origin.

| Isolate | Number of ESBL producers (%) | Positive genes detected by simplex PCR assays (n) | ESBL variant detected (n) | Other β-lactamase genes detected |
|---|---|---|---|---|
| *E. coli* hospitable (n=32) | 2.33 (1/43) | TEM, SHV, OXA-48 | TEM-1 (1) | SHV-85; OXA-181 |
| | 9.30 (4/43) | TEM, CTX-M-1 | CTX-M-15 (4) | TEM-1 |
| | 44.19 (19/43) | TEM | TEM-1 (19) | None |
| | 13.95 (6/43) | CTX-M-1 | CTX-M-15(6) | None |
| | 4.65 (2/43) | SHV | SHV-1(2) | None |
| *E. coli* community (n=11) | 4.65 (2/43) | TEM, CTX-M-1 | CTX-M-15 (2) | TEM-1 |
| | 13.95 (6/43) | TEM | TEM-1(6) | none |
| | 2.33 (1/43) | SHV | SHV-1 (1) | none |
| | 4.65 (2/43) | OXA-48 | OXA-181(2) | none |

M15 JQ686199. Two types of enzyme were detected for blaSHV: SHV-85 WP_063864713 (n = 1) and SHV-1 YES11730.1 (n = 3). Strains OXA-48 were OXA-181 variant with accession number HM992946.

## DISCUSSION

ESBL-producing bacteria are a major concern in community and hospital settings because of their epidemic spread and multidrug resistance. In fact, ESBLs are found in a large proportion of Gram-negative bacilli, but enterobacteria are the most incriminated organisms (Gniadkowski, 2001).

The present study, the first conducted in the Republic of Congo, detected 43 strains of *E. coli* producing ESBL on 89 strains tested, a prevalence of 48.31% between January 2016 and May 2017.

The obtained prevalence is relatively high compared to some studies, especially in Benin (22%) and Cameroon (14.3%) (Ahoyo et al., 2007; Gangoue et al., 2005). However, it is quite similar to that reported by Djukoué in Cameroon (45.3%) (Djuikoue et al., 2017).

The high prevalence of ESBL-producing *E. coli* isolates observed in the present study is probably a consequence of selection pressure due to inappropriate prescribing and misuse of broad-spectrum antibiotics in both hospital and community setting (dispensing without prescription, self-medication).

The rate of isolation of ESBL *E. coli* isolates was greater in the hospital (74.42%) than in the community (25.58%) with a statistically significant difference (*p*-value <0.05). These results corroborate with the literature on the epidemiology of ESBLs. The duration of hospitalization, the severity of the disease, the surgical intervention, the wearing of arterial or urinary catheters are the risk factors for the acquisition of ESBL in hospitalized patients (Bradford et al., 1997; Paterson, 2000).

Also, these species are distributed differently according

to the services and sites of sampling. Urinary tract infection is a common pathology in daily practice. The main bacterial species involved in this infection is *E. coli* since it represents 50 to 80% of the agents involved (Matute et al., 2004). This corresponds to the results obtained in the present study with a rate of 81.40%. This is related to the physiopathology of urinary tract infection, which is usually ascending, and there is a strong colonization of the perineum by Enterobacteriacae of digestive origin, and in particular *E. coli*. In addition, there are specific factors of uropathogenicity. Thus, *Escherichia coli* has adhesins, capable of binding the bacterium to the urinary epithelium and preventing its removal (Matute et al., 2004).

In the present study, most ESBL producers were collected from patients in the surgical ward and the pediatry than other reported services. In these wards, isolates are exposed to great antibiotic pressure. Furthermore, many of these patients are particularly vulnerable to infection because they are immunocompromised or have an easy avenue of access for bacteria (Xiong et al., 2002).

In addition, the problem related to ESBL is mainly the frequent presence of co-resistances making multiresistant strains (Touati et al., 2012).

Indeed, ESBLs are usually carried by large plasmids which also carry resistance genes to non-β-lactam antibiotic classes, such as aminoglycosides, quinolones and trimethoprim/sulfamethoxazole. As well, the use of these antibiotics contributes to the selection of producing strains from ESBL (Paterson and Bonomo, 2005).

The incidence of occurrence of resistances aminoglycoside has increased in recent years and particularly in producer of ESBL (Spanu et al., 2002). The resistance levels of these strains in the present study is more important for gentamycin (34.88%). Amikacin remains the most effective molecule with 90.7% of susceptible strains as reported in several studies (Gangoue et al., 2005; Touati et al., 2012).

The fluoroquinolones show a fairly good activity, the

overall sensitivity of the strains falling considerably in the case of strains producing ESBL. For the majority of strains, acquired resistance is the consequence of a mutation, which limits its diffusion (Larabi et al., 2003).

A very low activity was detected for trimethoprim/sulfamethoxazole (93.02%) and tetracyclines (with resistant strains). This correlates with other studies, where many ESBL producers are multi-resistant to non-β-lactam antibiotics, including fluoroquinolones and aminoglycosides (Livermore et al., 2007), Consequently, effective antibiotic therapy for treating these infections is limited to a small number of drugs, such as carbapenems and thus increasing the chance of resistance to carbapenems among the Enterobacteriaceae (Pitout, 2010).

As far as colistin is concerned, it is one of the molecules with the highest sensitivity levels on naturally occurring species sensitive (apart from *Proteus*). This is likely related to a lower use of this antibiotic in the current practice. This correlates with results of the susceptibility rate to this molecule were also reported by Nouria et al. (2016).

Carbapenems are the treatment of choice for severe infections caused by ESBL-producing enterobacteria (Nordmann et al., 2008). The resistance of enterobacteria to these molecules is still a marginal phenomenon as in the epidemiological data obtained for a large number of strains, with sensitivity percentages of 99 to 100% (Nordmann et al., 2008). In the present study, 9.3% strains had resistance to ertapenem and 100% were sensitive to imipenem. This decrease in sensitivity to ertapenem may be related to carbapenemase production since carbapenem activity may be compromised by the emergence of these enzymes (Munoz-price et al., 2013 ; Sekhri et al., 2010).

ESBLs are divided into five types: TEM, SHV, CTX-M, OXA-48, and others, based on the homogeneity of coding genes. Most ESBLs derived from plasmid-mediated penicillinases belonging to TEM or SHV familiies (Xue et al., 2012). Recently, the CTX-M group with a typical ESBL resistance phenotype but does not originate from TEM or SHV families have been described. The CTX-M group is a new family of plasmid-mediated ESBLs that preferentially hydrolyse cefotaxime (Xiong et al., 2002).

The prevalent genotypes vary in different contries such as the major genotypes TEM-10, TEM-12 and TEM-26 in U.S (Jacoby and Medeirof, 1991), TEM-10 and TEM-12 in United Kingdom. SHV-3, SHV-4 and TEM-4 in France. Previously, the most prevalent ESBLs in *E. coli* isolates from Korea as SHV-12 and CTX-M, as well as a prototype of Beta-lactams, TEM-1 (Xue et al., 2012). In Cameroon, Gangoue et al. (2005) report predominance of blaSHV-12 in Enterobacteriacea (Gangoue et al., 2005), as well as Benin, Ahoyo et al. (2007) reported a predominance of the *bla*SHV gene in *E. coli*.

TEM and SHV-type ESBLs remain more common in North American and in Africa. CTX-M type ESBLs have been mainly in South America, Eastern Europe, Japan and more recently in Spain, Kenya (Xue et al., 2012) and Algeria (Nouria et al., 2016).

In this study, it was determined that most of 32 *E. coli* isolates were prevalent TEM-type ESBLs. TEM was the main type of beta-lactamase and CTX-M was the second. SHV was detected in five isolates. In addition to OXA-48 was detected in 3 isolates. Another interesting point is that the results modify the current epidemiology of ESBL in Enterobacteriaceae, the CTX-M that represented the majority of ESBLs in any region of the world, both in hospitals and community settings, to such an extent that their spread of pandemic (Elhani, 2012).

The genetic diversity of ESBLs and carbapenemases can be attributed to genes already detected and the emergence of new clusters in our geographical area (TEM-1, SHV-1, SHV-85, CTX-M-15 and OXA-181) (Lonchel et al., 2012; Ouédraogo et al., 2016; Nouria et al., 2016).

## Conclusion

This can be the first study revealed the real existence of the genes encoding ESBL in *E. coli* isolates from the community and hospital patients in Brazzaville. A high prevalence (48, 31%) of ESBL *E. coli* isolates with predominance of the *bla*TEM gene. Fighting against this phenomenon is multidisciplinary and should integrate the rationalization of district compliance with the prescription of antibiotics, with hygiene measures. Justification of antibiotherapy policy and/or a restriction of the prescription of third-generation cephalosporin and even all beta-lactams would lead to a significant decrease in the frequency of ESBL.

Monitoring of antibiotic resistance of bacterial strains should be continuous and systematic to define therapeutic strategies adapted to local epidemiology data.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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