https://doi.org/10.46344/JBINO.2026.v15i02.06

AN OVERVIEW OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCING ENTEROBACTERIACEAE

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ABSTRACT

ESBL-producing Enterobacteriaceae are a growing concern in healthcare settings worldwide. These bacteria possess extended-spectrum beta-lactamases (ESBLs), enzymes that confer resistance to a broad range of antibiotics, including beta-lactams. This overview aims to provide a concise summary of the key aspects of ESBL-producing Enterobacteriaceae, including their prevalence, mechanisms of resistance, risk factors for infection, and implications for patient care. The rise in ESBL prevalence poses significant challenges in selecting effective antibiotics for treatment, leading to increased morbidity and mortality. Understanding the epidemiology and mechanisms of resistance of ESBL-producing Enterobacteriaceae is crucial for implementing appropriate control measures and developing strategies to combat further spread. Additionally, the molecular and phenotypic detection methods are briefly discussed, as they aid in identifying and characterizing these organisms. Overall, this overview highlights the urgent need for continued surveillance, research, and effective infection control strategies to counter the threats posed by ESBL-producing Enterobacteriaceae in healthcare settings.

***Keywords**: ESBL, Enterobacteriaceae, Resistance and Overview*

INTRODUCTION

Extended-spectrum β-lactamases (ESBLs) are a predominant cause of β-lactam resistance in Gram-negative bacilli (GNB). Incidences of infections caused by ESBLs producing GNB are increasing in prevalence worldwide, both in the healthcare as well as community settings, posing significant therapeutic challenges. ESBLs are most often a plasmid mediated heterogeneous group of β-lactamase enzymes, that confer resistance to a wide range of commonly used β-lactam antibiotics including third generation cephalosporins (e.g. ceftriaxone, cefotaxime and ceftazidime) as well as monobactams (aztreonam). Temoneira (TEM) and Sulfhydryl variable (SHV) type ESBLs used to be the dominant ESBL genotypes. However, in the past decade, the Cefotaximases (CTX-M) type ESBLs have become the most widely distributed and globally dominant genotypes[1].

The CTX-M type enzymes are a group of class A ESBLs that in general exhibit much higher levels of activity against cefotaxime and ceftriaxone than ceftazidime. The presence of CTX-M type ESBLs is often associated with co-resistance phenotypes in particular to fluoroquinolones and aminoglycosides, in addition to tetracycline, and trimethoprim/sulfamethoxazole co-resistance, which is commonly observed among TEM and SHV type ESBLs. The group of CTX-M type ESBLs currently constitutes more than 170 allelic variants, which cluster into five major groups based on sequence homologies. The five CTX-M groups are: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25. Each group consists of a number of particular variants with dominant variants being restricted in distribution to specific geographic areas, while few others are globally distributed. CTX-M-14 and CTX-M-15 were the most commonly isolated variants worldwide[1].

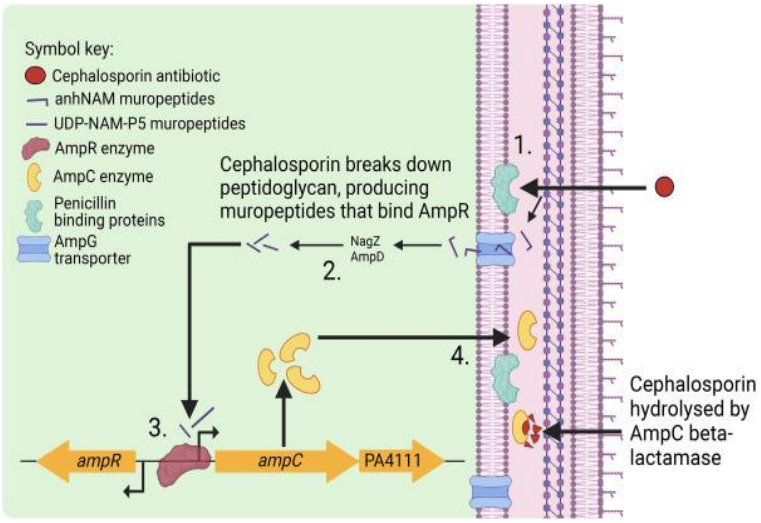

Fig. 1: Resistance mechanism of Cephalosporin [2].

Africa is a continent where antimicrobial resistance problems have not been illustrated adequately yet, due to the extremely low financial resources of many countries. Antibiotics, as well as other drugs, are lacking from several regions and thus common infections may be left untreated. The prevention of infections in African countries is, therefore, vital for the healthcare systems in Africa. Knowledge of the proportion of multidrug-resistant (MDR) bacteria in these countries could be helpful both to raise awareness of the need to prevent healthcare-associated infections and to improve clinical practice by guiding empirical antibiotic therapy [3].

The species which produces ESBLs are mostly *Klebsiella* species and *Escherichia coli* and it can be widely detected in other Gram–negative bacteria like;

- *Enterobacter*,
- *Salmonella*,
- *Citrobacter*,
- *Serratia marcescens*,
- *Proteus* species and
- *Pseudomonas aeruginosa*.

Unfortunately, the ESBL producers are associated with aforementioned bacteria with morbidity and mortality. It can be seen especially among patients on intensive care and high-dependency units. Additionally, the ESBL producing bacteria *Enterobacteriaceae* leads to a major health issue. It is because of the rate of infection is particularly high, and delays in the prescription of appropriate antimicrobial drug therapy for these infections are risk factors for poor prognosis and death [4].

Antibiotic resistance of bacteria is commonly seen in daily medical practice with multi-drug resistant Gram negative bacteria posing the greatest threat to human health. Beta lactam antibiotics are the most predominantly prescribed antibiotics to treat bacterial infections, especially in Nigeria hospitals. β-lactamases are major defense of Gram negative bacteria against β-lactam antibiotics [5]. Extended-spectrum β-lactamases (ESBLs) in Gram-negative organisms is now a major concern in *Enterobacteriaceae* worldwide [6]. Pathogenic bacteria evolve to resist the actions of antimicrobials through acquired and intrinsic mechanisms including production of β-lactamase enzymes, which inactivates antibiotic and decreases its therapeutic value. Extended-spectrum β-lactamases produced by many gram-negative bacteria, mostly *Enterobacteriaceae*, are able to hydrolyze penicillins, cephalosporin, and monobactams . They are mostly effective against a range of β-lactam drugs including ceftazidime, ceftriaxone, cefotaxime, and aztreonam. In many cases, resistance to these antibiotics is transferred among bacteria through gene transfer systems of mobile genetic elements carried in bacteria plasmids or transposons by bacterial recombination process that involves conjugation, transformation, and transduction. The world prevalence of community and hospital-acquired ESBL-PE is increasing tremendously. Bacteria harboring ESBL enzymes are currently the number one critical pathogens posing a major threat to

human health. The spread and dissemination of infections caused by ESBL-E are associated with increased morbidity and mortality, health care costs, the need for development of new wide-spectrum antimicrobials and lengthy hospital stay of infected patients [5].

This is because of a major decrease in therapeutic value of mostly used drugs as a result of resistance [7]. *Enterobacteriaceae* is a family of Gram-negative, facultatively anaerobic, non-spore-forming rods. Characteristics of this family include being motile, catalase positive, and oxidase negative; reduction of nitrate to nitrite; and acid production from glucose fermentation. However, there are also many exceptions. Currently, the family comprises 51 genera and 238 species. The number of species per genus ranges from 1 to 22. Twenty-two genera contain only one species, while seven genera have more than ten species [5]. Enterobacteriaceae are a large family of Gram-negative bacteria that includes a number of pathogens such as;

- *Klebsiella*,
- *Enterobacter*,
- *Citrobacter*,
- *Salmonella*,
- *Escherichia coli*,
- *Shigella*, *Proteus*,
- *Serratia* and other species.

These pathogens are present in the human intestinal tract and are a normal part of the gut flora. They are a common cause of urinary tract infections (UTIs), and some species can also cause diarrhoea. These pathogens can spread to the bloodstream resulting in life-threatening complication [5].

BACTERIA THAT PRODUCE ESBLS

The two most common bacteria that produce ESBLs are *Escherichia coli* (*E. coli*) and *Klebsiella pneumonia* both of which are found in the gut even when the individual is healthy (Falagas and Karageorgopoulos, 2019). Most *E. coli* strains and types are harmless, but some of them can cause infections leading to stomach pains and diarrhea. *Klebsiella pneumoniae* may make its way to other parts of the body, causing various infections like pneumonia and urinary tract infections or UTIs [8]. *E. coli* and *Klebsiella* infections are usually treated with common antibiotics like penicillins and cephalosporins. But, when these bacteria produce ESBLs, they become resistant to these antibiotics. An ESBL chemically breaks down and destroys its target antibiotic, making it useless against an infection [5].

EPIDEMIOLOGY

The overall pooled prevalence of ESBL-PE in Nigeria was 34.6% (95% CI 26.8 to 42.3%) and increased at a rate of 0.22% per year (p for trend=0.837) [9]. The meta-analysis computation derived an overall pooled ESBL PE prevalence in Nigeria of 34.6% (95% CI 26.8 to 42.3%). This number was derived from analysis of cumulative individual data on 5128 Enterobacteriaceae isolates. There is a dearth of data on population-based estimates of the incidence of ESBL in African countries, including Nigeria. To determine the Nigerian burden of ESBL in Enterobacteriaceae we extrapolated incidence based on a figure of 5.4–13.3 per 100 000 population using data from Thailand and Canada. With Nigeria's estimated population of 190 million, this

resulted in a rate of 1026–2527 per 100 000 population [10]. ESBL occurrence varied among bacteria assessed and was highest among Morganella morganii species (60.5%; 95% CI 18.2 to 102.7%) and lowest among Enterobacter species (20.8%; 95% CI 12.9 to 28.6%). However, excluding pooled ESBL-PE prevalence for organisms reported in less than five studies, estimates were highest among E. coli at 38.0% (95% CI 25.6 to 50.5%). We identified heterogeneity in the derived estimates. The proportion of ESBL-PE was highest in prospective studies, with a prevalence of 50.2% (95% CI 0.9 to 99.6%) compared with 33.4% (95% CI 25.4 to 41.4%) in cross-sectional studies. We identified rates of ESBL-PE ranging from 33.3 to 50.0%, depending on the diagnostic modalities deployed for detection. The highest rates of ESBL-PE were obtained from northcentral Nigeria, with a value of 63.2% (95% CI 49.3 to 77.1%) and were lowest in the southwest, with a prevalence of 28.2% (95% CI 20.7 to 35.7%). There was a lower pooled prevalence of 24.6% (95% CI 18.6 to 30.6%) from studies adjudged to be of 'good quality' (those with a rating of at least 70%) [11].

Stats of ESBL epidemiology are profoundly varied – all parts of the world have different rates of prevalence. In general terms; TEM-type ESBLs are predominantly reported in the United States, SHV-type ESBLs are most frequently isolated in Western Europe. CTX-M-type ESBLs have been detected in Australia, Latin America, Eastern Europe, and in specific countries such as Japan, Spain, & Kenya. Global epidemiology captures in major surveillance studies; Australian Group on Antimicrobial Resistance (AGAR) in Australia, European Antibiotic Resistance Surveillance System (EARSS) in European countries, Study for Monitor Antimicrobial Resistance Trends (SMART) in the United States of America and South-east Asia etc [11].

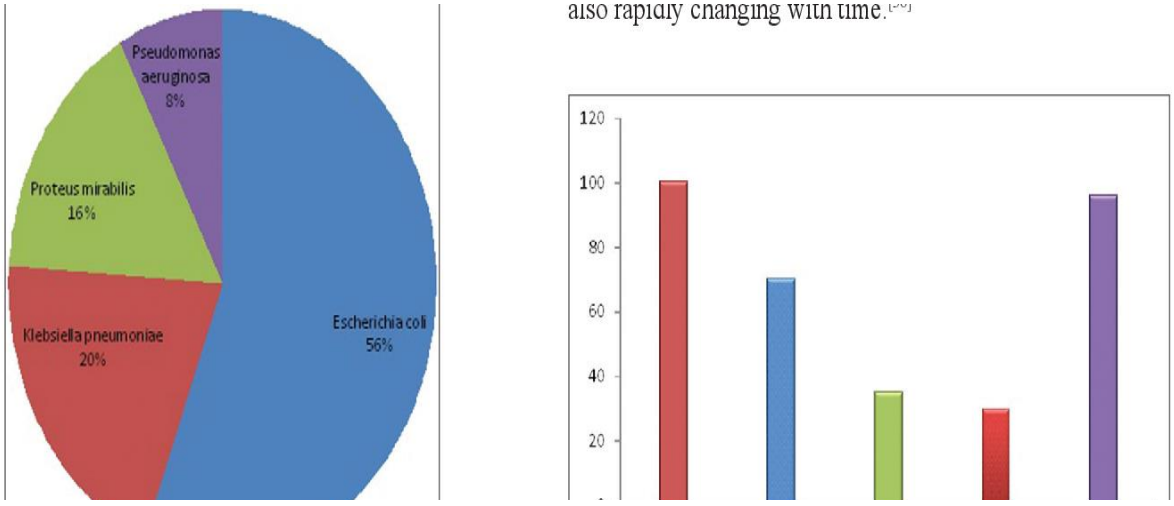

Fig. 2: Statistical representation of ESBL epidemiology[11].

CLASSIFICATION OF B-LACTAMASES

ESBLs were first reported in Germany in 1983. This followed introduction of broad spectrum 3G cephalosporins into clinical use. ESBLs have been reported in all parts of the world except Antarctica. ESBLs are derivatives of classic β-lactamases eg SHV-2 is derived from SHV-1. ESBLs are occasioned by single mutations in progenitor (parent) enzymes A mutation of few amino acids. ESBLs exhibit fundamental changes in substrate spectra, substrate profile, reactions to inhibitors & isoelectric point important distinguishing factors. Over 200 ESBLs are characterised & classified there is still no consensus on exact figure [12]. B-lactamases have been variously classified over time. Two commonly used classification schemes are; Ambler molecular classification system Bush-Jacoby-Medeiros functional classification system. The Ambler molecular system classifies β-lactamases on the basis of protein homology (amino acid similarities); 4 major classes (A, B, C & D). The Bush-Jacoby-Medeiros functional system classifies β- lactamases, on the basis of functional similarities/substrate and profile inhibitor profile;4 main groups (1, 2, 3 & 4). ESBLs are derived from group 2be β-lactamases; the `e' of 2be denotes the 'extended-spectrum' capability of the newly derived enzyme. ESBLs are quite diverse. Clinically important ESBLs are derived from 3 major types of classic beta-lactamases; TEM-, SHV-, & CTX-M-type β-lactamases. Temoniera – a Greek patient from whom this ESBL type was first isolated]13].

TYPES OF ESBLs ENZYMES

Most ESBLs are derivatives of native TEM or SHV genes by mutations that alter the amino acid configuration around the active site at selected loci within the gene giving rise to the extended spectrum phenotype. This extends the spectrum of beta lactam antibiotics susceptible to hydrolysis by these enzymes. An increasing number of ESBLs not of TEM or SHV lineage have been described, notably the CTX-M family, which represent plasmid acquisition of broad spectrum beta lactamases originally determined by chromosomal genes [14].

TEM ESBLs (TEMONEIRA)

The TEM-type ESBLs are derivatives of TEM-1 and TEM-2 (Rupp and Fey, 2020). Mutations within the *bla*TEM-1 structural gene, presumably by antibacterial selection has allowed this enzyme to expand the hydrolytic abilities to particular extended spectrum cephalosporins and aztreonam while maintaining its original hydrolytic capabilities to the ampicillin, carbenicillin and oxacillin. TEM-2 has same hydrolytic profile as TEM-1 but has different isoelectric point [15].

Currently more than 160 different TEM-type enzymes have been described and this is based upon the amino acid substitutions at 12 separate positions, acting alone or in concert with other structural gene mutations (Varghese and Rathi, 2023). Amino acid substitution in active site of enzyme is responsible for ESBL, which results in allowing excess amount of Oxy-imino-β-lactams. Most TEM type enzymes are ESBLs, but some are resistant to beta lactamase inhibitors and a few are

ESBLs and inhibitor resistant (Rupp and Fey, 2020). However TEM-1, TEM-2 and TEM-13 are not ESBLs [16].

The native SHV-1 beta lactamase is found primarily in *Klebsiella pneumoniae*. Specific mutations within the *bla*SHV-1 structural gene expand the hydrolytic abilities of SHV-1 to include the extended spectrum cephalosporins and monobactams. SHV type ESBL resembles 80% to type TEM SHV type are produced as a result of amino acid substitution at position 238 and 240. More than 100 SHV varieties are known. They are found worldwide with SHV-5 and SHV-12 being the most common [16].

CTX-M ESBLs (CEFOTAXIMASES)

CTX-M are new family of plasmid mediated ESBLs, that preferentially hydrolyse cefotaxime [17]. They represent examples of plasmid acquisition of beta lactamase gene found on the chromosome of *Kluyvera species*, a group of rarely pathogenic commensal organisms [18]. They have also been found in strains of *Salmonella enterica serovars typhimurium, Escherichia coli* and other species of *Enterobacteriaceae*. They are not closely related to TEM or SHV type beta lactamases in that they only show approximately 40% identity with these two commonly isolated beta lactamases [19]. More than 60 CTX-M enzymes have been described. These enzymes are clustered into five groups; CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25 [18].

OXA-TYPE ESBLs (OXACILLINASE)

OXA-type ESBLs have been found mainly in *Pseudomonas aeruginosa* isolates from Turkey and France [11]. The OXA-type ESBLs are derived from the OXA-type beta-lactamases and they differ from the TEM and SHV enzymes in that they belong to molecular class D and functional group 2d. The native OXA-type beta-lactamases confer resistance to ampicillin and cephalothin [15]. They are characterized by their high hydrolytic activity against oxacillin and cloxacillin and the fact that they are poorly inhibited by clavulanic acid. Amino acid substitutions in the OXA enzymes results in the ESBL phenotype [15]. There is as little as 20% sequence homology among some of the members of the OXA-ESBL family. However, recent additions to this family show some degree of homology to one or more of the existing members of the OXA beta-lactamase family. Some confer resistance predominantly to ceftazidime, but OXA-17 confers greater resistance to cefotaxime and cefepime than it does resistance to ceftazidime [15].

OTHER ESBL TYPES

Other plasmid-mediated ESBLs, such as PER (Pseudomonas extended resistance), VEB (Vietnamese extended-spectrum beta-lactamase), GES, and IBC beta-lactamases, have been described but are uncommon. They have been found mainly in *Pseudomonas aeruginosa* and at a limited number of geographic sites. PER-1-Beta lactamase was first described in strains of *Pseudomonas aeruginosa* isolated from patient in Turkey [20]. Later, it was also found among isolates of *Salmonella enterica serovars typhimurium* and *Acinetobacter baumanii*. The PER-1 beta lactamase is not related to TEM-1 or SHV-1-derieved enzymes. They degrade cephems, monobactams, in addition to

conferring high level resistance to anti-*Pseudomonas* beta lactam [11]. Another enzyme somewhat related to PER-1 is the VEB-1 beta lactamase. VEB-1 was first isolated in a single isolate of *Escherichia coli* in a patient from Vietnam, but was later on found in *Pseudomonas aeruginosa* isolate from a patient in Thailand. CME-1 is another related enzyme which was isolated from *Chryseobacterium meningosepticum* [21]. TLA-1 was identified in an *Escherichia coli* isolate from a patient in Mexico. The PER-1, PER-2, VEB-1, CME-1 and TLA-1 beta lactamases are related but only show 40-50% homology [21]. These enzymes all confer resistance to oxyimino-cephalosporins, especially ceftazidime and aztreonam. SFO-1 is another enzyme which is highly related to class A beta lactamases from *Serratia fonticola* [11]. GES-1 is another uncommon ESBL enzyme that is not closely related to any other plasmid mediated beta lactamases but does show 36% homology to carbenicillinase from *Proteus mirabilia* [11].

MODE OF TRANSMISSION OF ESBL

As with other bacteria, ESBL-producing bacteria can spread from person to person or by contact with a contaminated surface. The infections can be contracted by simply by shaking hands with an infected person or by touching soiled objects that have not been cleaned thoroughly. The spread of disease-causing bacteria is especially common in health care settings, where their numbers in the surroundings are very high [22]. Infections due to ESBL-producing bacteria are easily spread by healthcare professionals like doctors and nurses who regularly come in contact with contaminated surfaces. People who are ill or are taking large doses of antibiotics are at a higher risk of getting infected with ESBL-producing bacteria. People receiving treatment in hospitals or care homes are also easily exposed to infections, particularly if they have open wounds, drainage tubes, or catheters [23].

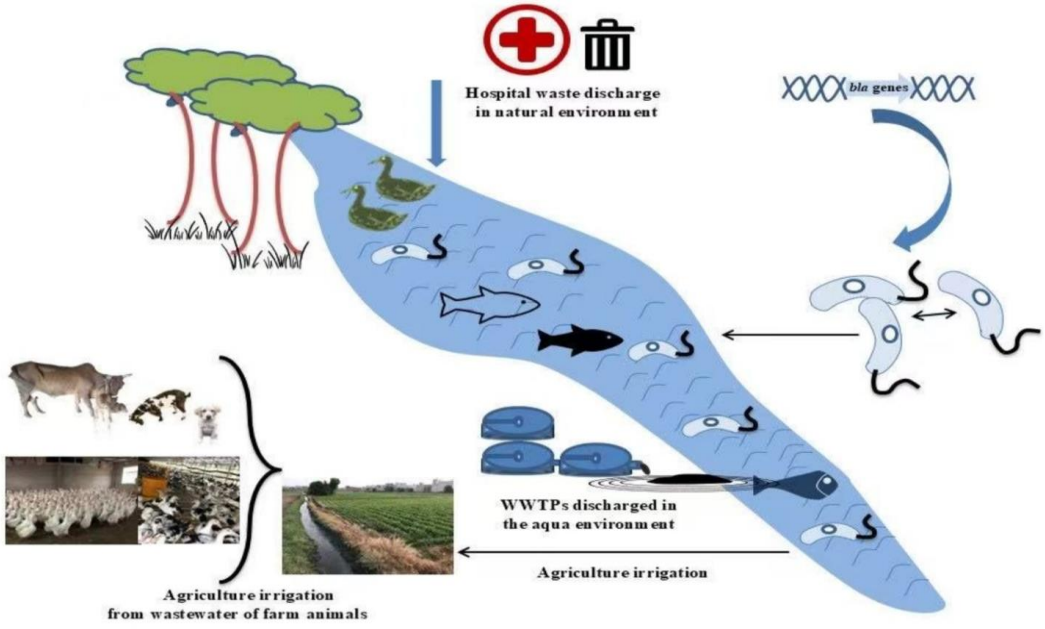

Fig. 3: An illustrative diagram of mode of transmission of ESBL [24]

CLINICAL SIGNIFICANCE OF ESBL-PRODUCING ENTEROBACTERIACEAE (ESBL-PE)

ESBL-producing Enterobacteriaceae (ESBL-PE) cause significant mortality and morbidity globally (Rodríguez-Baño and Pascual, 2019). ESBL-PE cause a range of infections including uncomplicated UTIs, life- threatening bacteraemia, URTIs, gastroentritis, and colonising wound infections [22]. Mortality of patients with ESBL positive sepsis is significantly higher than those with ESBL negative sepsis, up to 30% of GNB-caused sepsis is fatal. Are implicated in large scale outbreaks in hospital or community settings. Cause localised or institutionalised outbreaks [25]. Infections caused by ESBL-PE are associated with rising healthcare cost. Decreased productivity as a consequence of prolonged hospitalisation. ESBL-PE are associated with increasing episodes of clinical treatment failure. ESBL producing organisms have important therapeutic and clinical ramifications for patients from whom they are isolated. ESBL-PE pose significant public health risks. ESBL-PE pose serious infection control challenges. ESBL production in Enterobacteriaceae has been a consequence of widespread use of broad-spectrum antibiotics in hospital settings. Increasing prevalence is reported in isolates recovered from community-based patients. ESBLs are transferrable via conjugative plasmids thus dissemination of resistance genes among bacterial populations can occur and spread in larger geographic regions. Treatment of ESBL-PE involves a combination of antibiotics, some of which have undesirable side effects including nephrotoxicity [22].

SYMPTOMS OF INFECTIONS DUE TO ESBL-PRODUCING BACTERIA

Symptoms in such infections mainly depend on the type of bacteria that cause the infection and the affected area of your body [26]. The most commonly diagnosed sites of infection are the gut and the urinary tract, although the lungs, open wounds, and blood can also get infected with ESBL-producing bacteria. Symptoms of a UTI may include:

- Burning sensation or pain when urinating
- Pressure in the lower belly
- Cloudy or reddish urine
- Urge to urinate often

Infections due to ESBL-producing bacteria in the gut may present with symptoms such as:

- Stomach cramps
- Vomiting
- Diarrhea
- Loss of appetite

Infections that has spread to the blood may be accompanied by symptoms such as: symptoms such as:

- Fever
- Aches and pains
- Weakness
- Chills
- Disorientation [22].

RISK FACTORS FOR ESBL-PRODUCING ENTEROBACTERIACEAE

Significant risk factors among underlying diseases included cerebrovascular disease and urological disease. Additionally, cancer, cardiovascular disease, cerebrovascular disease and major surgery

within 60 days were also significantly associated with ESBL production [27]. Among medical devices, urinary catheterization, intubation/ tracheostomy, nasogastric tube, central venous catheter, drain, and artificial organ were risk factors for ESBL production [28]. Risk factors for ESBL infection include age, comorbidities, recurrent urinary tract infections (UTI), intensive care unit (ICU) stay, previous use of antibiotics, and colonization with ESBL [29].

Risk factors for infections with ESBL-PE in healthcare- or community-acquired infections include; Previous use of antibiotics including broad spectrum antibiotics e.g. 3GC cephalosporins; Recent or prolonged hospital admissions including admissions to ICU; Recurrent UTIs; Empiric antibiotic therapy; Increased age; female gender; Institutionalised residential care e.g. nursing homes; Intravenous therapy; International travels to areas of established endemicity e.g. India subcontinent, the Middle East and Africa; Immunosuppressive chemotherapy; Invasive procedures-indwelling urinary catheters; central venous catheter, and Underlying comorbidities such as chronic renal insufficiencies, haemodialysis, liver disease, diabetes mellitus, malignancy, hypertension, heart disease, neutropenia, and HIV infection [30].

DETECTION OF ESBL PRODUCING ENTEROBACTERIACEAE IN CLINICAL ISOLATE

ESBL-PE can be detected through the use of both genotypic and phenotypic techniques. Phenotypic testing is a 2 steps process, which includes;

- Screening
- Confirmation

Screening is a process aimed at excluding potential ESBL-producing isolates by testing for resistance or reduced susceptibility to 3GC [third generation of cephalosporins]. Screening is carried out by using cefotaxime, cefpodoxime, ceftazidime, and aztreonam discs. Multiple 3GC agents reliably improves sensitivity by offering wider ESBL substrate base. Confirmation is the second step, which tests for synergy between 3GC cephalosporins & clavulanates (synergy between β-lactams and β-lactams-clavulanate combinations). It is also known as DDST (double disc synergy test).

A disc zone diameter difference of ≥5 mm between a cephalosporin and its respective cephalosporin-clavulanate is taken as a phenotypic confirmation of ESBL production e.g. an ESBL-producer tested against ceftazidime produces these resistance zones: ceftazidime zone = 16; ceftazidime-clavulanic acid zone = 21 Automated (Vitek 2 systems) MBD Automated microbroth dilution - growth at or above screening concentrations (breakpoint) may indicate production of ESBL (that is, for E. coli and K. pneumoniae, MIC ≥ 2 µg/mL for ceftriaxone, ceftazidime, aztreonam, or cefpodoxime). E-test, microScan panels and other discs-based methods are also used [30].

DESCRIPTION OF THE ESBL DETECTION TESTS

1) Double-disk synergy test

The first test specifically designed to detect ESBL production in *Enterobacteriaceae* was the double-disk synergy test (DDST) (Larramendy *et al.*, 2020). It was initially designed to differentiate between cefotaxime-resistant strains, i.e., those overproducing

cephalosporinase, and those producing ESBLs. The test is performed on agar with a 30-µg disk of cefotaxime (and/or ceftriaxone and/or ceftazidime and/or aztreonam) and a disk of amoxicillin–clavulanate (containing 10 µg of clavulanate) positioned at a distance of 30 mm (centre to centre), i.e., at the distance provided by several types of disk-dispenser [30]. The test is considered as positive when a decreased susceptibility to cefotaxime is combined with a clear-cut enhancement of the inhibition zone of cefotaxime in front of the clavulanate-containing disk, often resulting in a characteristic shape-zone referred to as 'champagne-cork' or 'keyhole'. give several examples of positive DDSTs for different enzymes and Enterobacteriaceae species [31]. It has been shown to work well with a wide range of Enterobacteriaceae species and ESBL types, and it is generally regarded as a reliable method for the detection of ESBLs, although it is sometimes necessary to adjust the disk spacing [31]. It is important to note that reducing the distance between the clavulanate-containing disk and the third-generation cephalosporin disk (e.g., to 20 mm) significantly improves the test sensitivity [32].

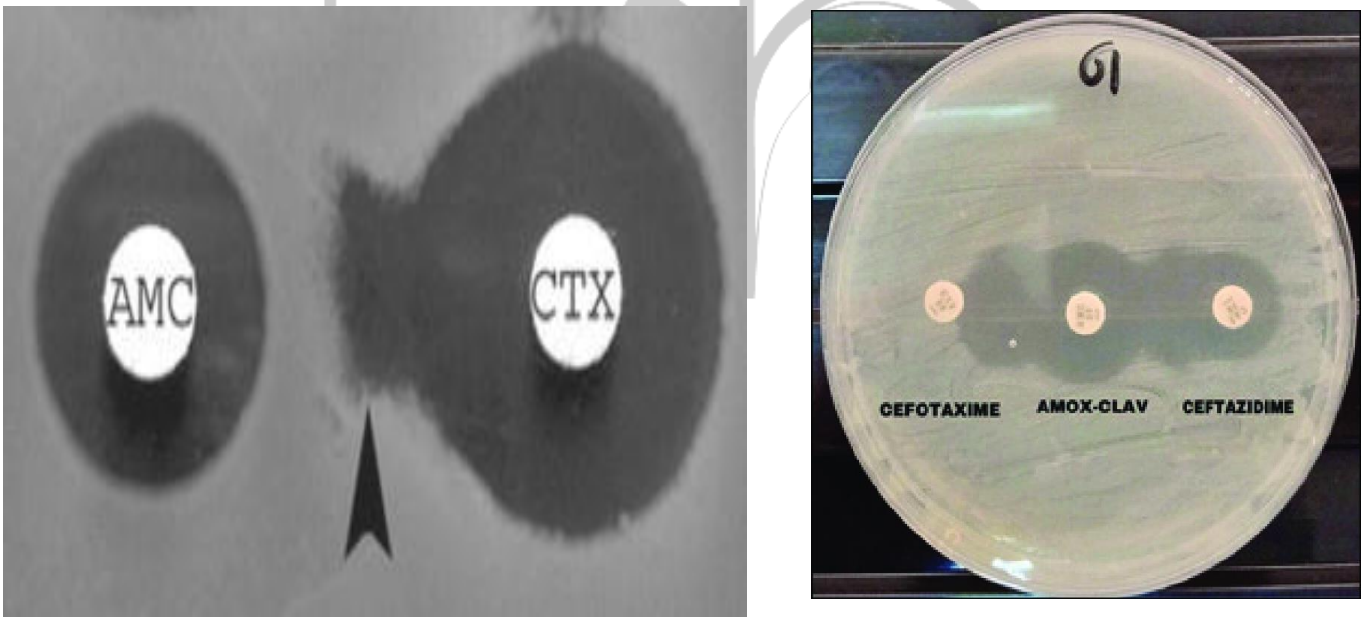

Fig. 4: ESBL detection -Double disk synergy test[18].

2) ESBL Etests

ESBL Etests have been developed in order to quantify the synergy between extended-spectrum cephalosporins and clavulanate. The Etests called CT/CTL, TZ/TZL and PM/PML are two-sided strips containing gradients of cefotaxime (CT), or ceftazidime (TZ) or cefepime (PM), either alone (at one end of the strip), or combined with clavulanate 4 mg/L (on the other end). The ESBL test is considered as positive when the MIC value of the tested drug is reduced by more than three doubling dilution steps (MIC ratio ≥8) in the presence of clavulanate [31]. The test is also considered as positive when there is either: (a) a rounded zone (phantom zone) just below the lowest concentration of CTL, TZL or PML gradients, or (b) a deformation of the CT, TZ or PM inhibition ellipse at the tapering end. The presence of a phantom zone or an ellipse deformation indicates ESBL production. Interpreting results of the ESBL Etest strips is delicate and requires training. In a recent study, it has been reported that laboratories may fail to interpret correctly the inhibition ellipse in c. 30% of cases [33]. In addition, ESBL detection by Etest may fail when the MIC values for cephalosporins fall outside the range of MICs available on the test strip [34].

3) Combination disk method

Several manufacturers have developed ESBL detection tests based on the combination disk method. The principle of this method is to measure the inhibition zone around a disk of cephalosporin and around a disk of the same cephalosporin plus clavulanate. Depending on the disk type, a difference of ≥5 mm between the two diameters (i.e., corresponding to a two-fold dilution), or a zone expansion of 50% are considered as indicating ESBL production[35]. The test is easy to perform and its interpretation is straightforward. Sensitivity and specificity for this method were first reported to be 96% and 100%, respectively. Carter *et al.* evaluated the performance of the Oxoid cefpodoxime 10 ng ± 1 µg clavulanate combination disks to distinguish ESBL producers from AmpC overproducers and Klebsiella oxytoca isolates overexpressing K1 enzyme. The presence of clavulanate enlarged the zone of inhibition by ≥5 mm for all 180 ESBL-producing organisms, and by <1 mm for AmpC overproducers and *K. oxytoca* isolates overexpressing K1 enzyme [35].

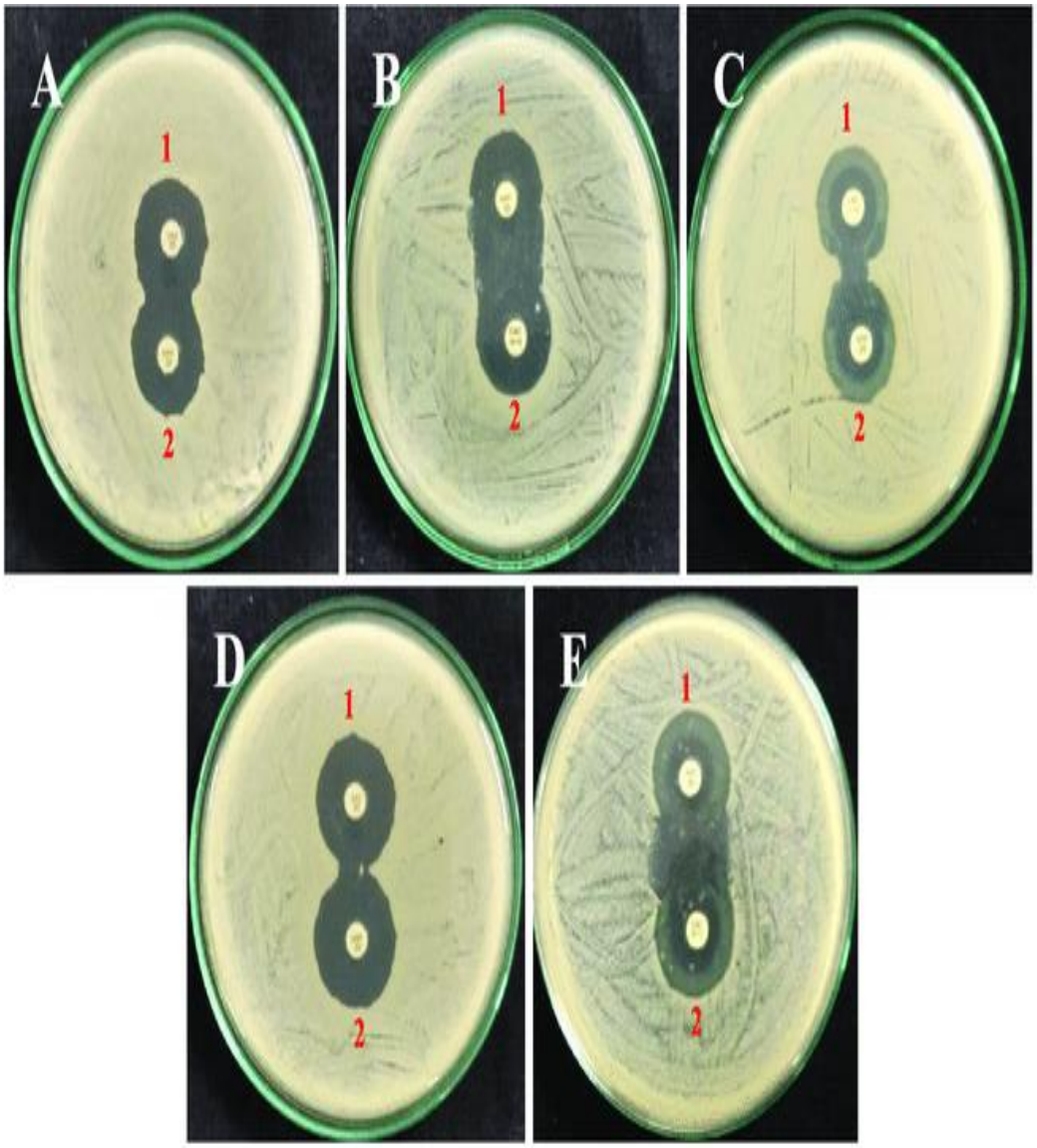

Fig. 5: Double disc combination method for ESBL identification. The ceftazidime expanded into the mouth of the amoxiclav (augmentin) was clearly observed in the plates (A) *E. coli*, (B) *P. mirabilis*, (C) *P. aureginosa*, (D) *K. pneumoniae* and (E) *Acinetobacter spp*. [35].

PREVENTING GROWING THREATS OF ESBL-MEDIATED ANTIMICROBIAL RESISTANCE

What should be done to curb increasing threats pose by ESBL- mediated antibiotic resistance; Robust antibiotic stewardship appropriate use of antibiotics. Effective infection control measures in hospitals effective preventive measures to curb transmission; contact precautions, hand hygiene, disinfections of inanimate objects, surfaces, medical devices in healthcare facilities public education antibiotic resistance awareness campaign [36]. Controlling use of antibiotics in food chains. Control and regulation of antibiotic use in agriculture. Immunization, preventative and indirect development of newer, potent antibiotics against emerging multidrug resistant bacteria [36]. Timely detection, and reporting of ESBL producing bacteria by medical laboratories. Instituting infection control measures in institutionalised care settings eg nursing homes. Active screening for multi-drug resistant Enterobacteriaceae. Classifying ESBL-PE as notifiable infections. Thorough hand-washing and sanitizing are the best ways to control the spread of infections caused by ESBL-producing bacteria [36].

The hands must be clean after using a bathroom and after touching exposed foods like raw meat. If you are around someone with an infection due to ESBL-producing bacteria, special care should be taken to clean frequently touched objects and surfaces. A healthcare provider should then maintain high standards of cleanliness to prevent care staff from spreading the infection. Also, if you are infected or are caring for someone with an infection caused by ESBL-producing bacteria, follow these good hygiene practices:

- Clean hands frequently
- Avoid sharing food or other personal items
- Wash laundry with detergent and warm water
- Avoid exposure to public spaces

In some cases of serious infections, you may need to be isolated or quarantined while receiving treatment in a hospital (Sharma *et al.*, 2023). This step is not necessary if you are recovering at home. General precautions you can take to control the spread of ESBL-producing bacteria are:

- Maintaining proper hand hygiene
- Routinely disinfecting surfaces, especially in the kitchen and bathrooms
- Avoiding touching the face and mouth
- Taking prescribed antibiotics exactly as directed
- Most infections due to ESBL-producing bacteria can be easily treated with a course of the right antibiotics. Of the many antimicrobial medicines, your doctor will prescribe you drugs based on the bacterial strain and site of infection.
- Once the infection has been treated, it is important to follow good hygiene practices so you don't develop another treatment-resistant infection [37]

TREATMENT OF INFECTIONS CAUSED BY ESBL-PRODUCING ENTEROBACTERIACEAE

Therapeutic options are very limited. Treatment usually involves a combination of drugs. These are usually the expensive, last line of antibiotics; Carbapenems (e.g

meropenem, ertapenem) Fosfomycin.β-lactam/β-lactam-inhibitor combination drugs (e.g Amoxicillin-clavulanate, piperacillin-tazobactam etc) – supporting evidence from clinical studies is, however, controversial [38]. Limitation of therapeutic drugs is also compounded by other factors such as; Site of infection, Severity of infection, Renal or liver functions of a patient, Age, Pregnancy or lactation status, Other medications the patient may be taking. Even though ESBL-producing bacteria have defense mechanisms against several commonly used antibiotics, many other available drugs can be used here. But, if you have a severe infection, you might need to be hospitalized for treatment with intravenous (IV) antibiotics [38]. Carbapenems are the most commonly prescribed antimicrobial drugs for treating infections caused by highly resistant ESBL-producing bacteria. Other prescribed medications may include:

- Ceftriaxone
- Cefepime
- Cefotaxime or aztreonam
- Cefpodoxime

GENERAL MANAGEMENT RECOMMENDATIONS

Treatment recommendations in this guidance document assume that the causative organism has been identified and that in vitro activity of antibiotics is demonstrated. Assuming two antibiotics are equally effective, safety, cost, convenience, and local formulary availability are important considerations in selecting a specific agent. The panel recommends that infectious diseases specialists and physician or pharmacist members of the local antibiotic stewardship program are involved in the management of patients with infections caused by antimicrobial-resistant organisms [39]. In this study, the term complicated urinary tract infection (cUTI) refers to UTIs occurring in association with a structural or functional abnormality of the genitourinary tract, or any UTI in an adolescent or adult male. In general, the panel suggests cUTI be treated with similar agents and for similar treatment durations as pyelonephritis. For cUTI where the source has been controlled (eg, removal of a Foley catheter) and ongoing concerns for urinary stasis or indwelling urinary hardware are no longer present, it is reasonable to select antibiotic agents and treatment durations similar to uncomplicated cystitis [38].

Empiric Therapy

Empiric treatment decisions should be guided by the most likely pathogens, severity of illness of the patient, the likely source of the infection, and any additional patient-specific factors (e.g. severe penicillin allergy, chronic kidney disease). When determining empiric treatment for a given patient, clinicians should also consider:

1) previous organisms identified from the patient and associated antibiotic susceptibility data in the last 6 months,
2) antibiotic exposures within the past 30 days, and
3) local susceptibility patterns for the most likely pathogens.

Empiric decisions should be refined based on the identity and susceptibility profile of the pathogen [40].

Duration of Therapy and Transitioning to Oral Therapy

Recommendations on durations of therapy are not provided, but clinicians are advised that the duration of therapy should not differ for infections caused by organisms with resistant phenotypes compared to infections caused by more susceptible phenotypes. After antibiotic susceptibility results are available, it may become apparent that inactive antibiotic therapy was initiated empirically. This may impact the duration of therapy. For example, cystitis is typically a mild infection [37]. If an antibiotic not active against the causative organism was administered empirically for cystitis, but clinical improvement nonetheless occurred, the panelists agree that it is generally not necessary to repeat a urine culture, change the antibiotic regimen, or extend the planned treatment course. However, for all other infections, if antibiotic susceptibility data indicate a potentially inactive agent was initiated empirically, a change to an active regimen for a full treatment course (dated from the start of active therapy) is recommended. Additionally, important host factors related to immune status, ability to attain source control, and general response to therapy should be considered when determining treatment durations for antimicrobial-resistant infections, as with the treatment of any bacterial infection. Finally, whenever possible, oral step-down therapy should be considered, particularly if the following criteria are met:

1) susceptibility to an appropriate oral agent is demonstrated,
2) the patient is hemodynamically stable,
3) reasonable source control measures have occurred, and
4) concerns about insufficient intestinal absorption are not present [38].

RECOMMENDATION

A carbapenem is preferred for the treatment of infections outside of the urinary tract caused by ESBL-E. After appropriate clinical response is achieved, transitioning to oral fluoroquinolones or trimethoprim-sulfamethoxazole should be considered, if susceptibility is demonstrated [40].

CONCLUSION

The problem of ESBL-producing Enterobacteriaceae infection is no longer limited to hospital-acquired infections, while some previous studies revealed that ESBL production was likely to be a surrogate of healthcare exposure. The presence of chronic illness was risk factors for the acquisition of ESBL, such as cerebrovascular disease. In addition, antimicrobial treatments with aminoglycoside, oxazolidinone, tetracycline, fluoroquinolone, trimethoprim or sulfamethoxazole, second- and fourth generation cephalosporin were potent risk factor for ESBL producing Enterobacteriaceae.

REFERENCES

1. Zeynudin, A., Pritsch, M., Schubert, S., Messerer, M., Liegl, G., Hoelscher, M., Belachew, T. and Wieser, A. (2019). Prevalence and Antibiotic Susceptibility Pattern of Ctx-M Type Extended-Spectrum B-Lactamases among Clinical Isolates of Gram-Negative Bacilli in Jimma, Ethiopia. *BMC Infectious Disease,* **18**(1): 524.

2. Boys, M. T. H., & Pletzer, D. (2025). A review of recently discovered mechanisms of cephalosporin resistance in Pseudomonas aeruginosa. *International journal of antimicrobial agents*, *66*(2), 107527.
3. Tansarli, G.S., Poulikakos, P., Kapaskelis, A. and Falagas, M.E., 2019. Proportion of extended-spectrum β-lactamase (ESBL)-producing isolates among Enterobacteriaceae in Africa: evaluation of the evidence—systematic review. *Journal of Antimicrobial Chemotherapy*, *69*(5), pp.1177-1184.
4. Saravanan, M., Ramachandran, B. and Barabadi, H., 2019. The prevalence and drug resistance pattern of extended spectrum β–lactamases (ESBLs) producing Enterobacteriaceae in Africa. *Microbial pathogenesis*, *114*, pp.180-192.
5. Ogefere, H.O., Aigbiremwen, P.A. and Omoregie, R., 2021. Extended-spectrum beta-lactamase (ESBL)–producing Gram-negative isolates from urine and wound specimens in a tertiary health facility in southern Nigeria. *Tropical Journal of Pharmaceutical Research*, *14*(6), pp.1089-1094.
6. Raji, M. A., Jamal, W., Ojemeh, O. and Rotimi, V. O. (2019). Sequence Analysis of Genes Mediating Extended-Spectrum Beta-Lactamase (Esbl) Production in Isolates of Enterobacteriaceae in a Lagos Teaching Hospital, Nigeria. *BMC Infectious Diseases,* **15**(1): 259.
7. Onduru, O.G., Mkakosya, R.S., Aboud, S. and Rumisha, S.F., 2021. Genetic determinants of resistance among ESBL-producing enterobacteriaceae in community and hospital settings in east, central, and Southern Africa: A systematic review and meta-analysis of prevalence. *Canadian Journal of Infectious Diseases and Medical Microbiology*, *2021*, pp.1-9.
8. Paterson, D.L. and Bonomo, R.A., 2021. Extended-spectrum β-lactamases: a clinical update. *Clinical microbiology reviews*, *18*(4), pp.657-686.
9. Musa, B.M., Imam, H., Lendel, A., Abdulkadir, I., Gumi, H.S., Aliyu, M.H. and Habib, A.G., 2020. The burden of extended-spectrum β-lactamase-producing Enterobacteriaceae in Nigeria: a systematic review and meta-analysis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *114*(4), pp.241-248.
10. Skogberg, K., Lyytikäinen, O., Ollgren, J., Nuorti, J.P. and Ruutu, P., 2019. Population-based burden of bloodstream infections in Finland. *Clinical Microbiology and Infection*, *18*(6), pp.E170-E176.
11. Sawatwong, P., Sapchookul, P., Whistler, T., Gregory, C.J., Sangwichian, O., Makprasert, S., Jorakate, P., Srisaengchai, P., Thamthitiwat, S., Promkong, C. and Nanvatthanachod, P., 2019. High burden of extended-spectrum β-lactamase–producing Escherichia coli and klebsiella pneumoniae bacteremia in older adults: A seven-year study in two rural Thai provinces. *The American journal of tropical medicine and hygiene*, *100*(4), p.9431
12. Ghafourian, S., Sadeghifard, N., Soheili, S. and Sekawi, Z., 2020. Extended spectrum beta-lactamases: definition, classification and epidemiology. *Current issues in molecular biology*, *17*(1), pp.11-22.
13. Bush, K. and Jacoby, G.A., 2020. Updated functional classification of β-lactamases. *Antimicrobial agents and chemotherapy*, *54*(3), pp.969-976.
14. Aboderin, O. A., Adefehinti, O., Odetoyin, B. W., Olotu, A. A., Okeke, I. N. and Adeodu,

O. O. (2020). Prolonged Febrile Illness Due to Ctx-M-15 Extended-Spectrum B-Lactamase-Producing Klebsiella Pneumoniae Infection in Nigeria. *Africa Journal of Labouratory Medicine,* **1**(1): 16.

15. Rupp, M.E. and Fey, P.D., 2020. Extended spectrum β-lactamase (ESBL)-producing Enterobacteriaceae: considerations for diagnosis, prevention and drug treatment. *Drugs*, *63*, pp.353-365.
16. Varghese, S. and Rathi, M.A., 2023. Genotypic Characterization Of Virulence Factor Afa, Papc, Sfa And Molecular Detection Of Bla TEM, Bla SHV, Bla CTX-M Genes According To Phytogenetic Group In Extended Spectrum B-Lactamase (ESBL) Escherichia Coli Strains Isolated From Urinary Tract Infect. *Journal of Survey in Fisheries Sciences*, pp.978-986.
17. Seck, A., Ndiaye, B., Diop, A., Ndao, M., Fall, C., Dieng, A., Diallo, T.A., Mahou, C. and Dubrous, P., 2023. Prevalence and Resistance Profile of Muenchen Cefotaximase (CTX-M) Group 1 Extended Spectrum Beta-Lactamase (ESBL)-Producing Uropathogenic Escherichia coli Strains in Dakar, Senegal. *Open Journal of Medical Microbiology*, *13*(2), pp.137-145.
18. Okoro, A. E., Akujobi, C. N., Aghanya, N. I., Ukibe, S. N., Nnemelu, P. O., Okwesileze, C. V., Ezeama, J. C., Akuakaloma, I. M. and Ajuba, I. M. (2023). Molecular Detection of Cefotaximase Munich (Ctx-M) Resistant Acinetobater Baumannii in a Tertiarycare Hospital in Southeast Nigeria. *Journal of Biomedical Investigation,* **11**(1): 41-46.
19. Higgins, O., Chueiri, A., O'Connor, L., Lahiff, S., Burke, L., Morris, D., Pfeifer, N.M., Santamarina, B.G., Berens, C., Menge, C. and Caniça, M., 2023. Portable Differential Detection of CTX-M ESBL Gene Variants, bla CTX-M-1 and bla CTX-M-15, from Escherichia coli Isolates and Animal Fecal Samples Using Loop-Primer Endonuclease Cleavage Loop-Mediated Isothermal Amplification. *Microbiology Spectrum*, *11*(1), pp.e03316-22.
20. Yassara, S., Zeouk, I., Jaouhar, S., Sbiti, M., & Bekhti, K. (2025). Extended-spectrum beta-lactamases: definition, history, an update on their genetic environment and detection methods. *Journal of Medical Microbiology*, *74*(6), 002033.
21. Bennett, J. W., Robertson, J. L., Hospenthal, D. R., Wolf, S. E., Chung, K. K., Mende, K. and Murray, C. K. (2020). Impact of Extended Spectrum Beta-Lactamase Producing Klebsiella Pneumoniae Infections in Severely Burned Patients. *Journal of the American College of Surgeons,* **211**(3): 391-399.
22. Frank Wolf, M., Abu Shqara, R., Naskovica, K., Zilberfarb, I.A., Sgayer, I., Glikman, D., Rechnitzer, H., Fleisher Sheffer, V. and Bornstein, J., 2021. Vertical transmission of extended-spectrum, beta-lactamase-producing Enterobacteriaceae during preterm delivery: a prospective study. *Microorganisms*, 9(3), p.506.
23. Schmitt, K., Kuster, S.P., Zurfluh, K., Jud, R.S., Sykes, J.E., Stephan, R. and Willi, B., 2021. Transmission chains of extended-spectrum beta-lactamase-producing enterobacteriaceae at the companion animal veterinary clinic–household interface. *Antibiotics*, *10*(2), p.171.
24. Zhang, S., Yang, J., Abbas, M., Yang, Q., Li, Q., Liu, M., ... & Cheng, A. (2025). Threats across boundaries: the spread of ESBL-positive Enterobacteriaceae bacteria and its challenge to the "one health"

concept. *Frontiers in microbiology*, *16*, 1496716.

25. Ramphal, R. and >, P.G.A., 2019. Extended-spectrum β-lactamases and clinical outcomes: current data. *Clinical infectious diseases*, *42*(Supplement_4), pp.S164-S172.
26. Peirano, G. and Pitout, J.D., 2019. Extended-spectrum β-lactamase-producing Enterobacteriaceae: update on molecular epidemiology and treatment options. *Drugs*, *79*, pp.1529-1541.
27. Massart, N., Camus, C., Benezit, F., Moriconi, M., Fillatre, P. and Le Tulzo, Y., 2020. Incidence and risk factors for acquired colonization and infection due to extended-spectrum beta-lactamase-producing Gram-negative bacilli: a retrospective analysis in three ICUs with low multidrug resistance rate. *European Journal of Clinical Microbiology & Infectious Diseases*, *39*, pp.889-895.
28. Nakai, H., Hagihara, M., Kato, H., Hirai, J., Nishiyama, N., Koizumi, Y., Sakanashi, D., Suematsu, H., Yamagishi, Y. and Mikamo, H., 2019. Prevalence and risk factors of infections caused by extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae. *Journal of Infection and Chemotherapy*, *22*(5), pp.319-326.
29. Ben-Ami, R., Rodríguez-Baño, J., Arslan, H., Pitout, J. D. D., Quentin, C., Calbo, E. S., Azap, Ö. K., Arpin, C., Pascual, A., Livermore, D. M., Garau, J. and Carmeli, Y. (2019). A Multinational Survey of Risk Factors for Infection with Extended-Spectrum B-Lactamase-Producing Enterobacteriaceae in Nonhospitalized Patients. *Clinical Infectious Diseases,* **49**(5): 682-690.
30. Larramendy, S., Deglaire, V., Dusollier, P., Fournier, J.P., Caillon, J., Beaudeau, F. and Moret, L., 2020. Risk factors of extended-spectrum beta-lactamases-producing Escherichia coli community acquired urinary tract infections: a systematic review. *Infection and Drug Resistance*, pp.3945-3955.
31. Thomson, K.S. and Sanders, C.C., 2021. Detection of extended-spectrum beta-lactamases in members of the family Enterobacteriaceae: comparison of the double-disk and three-dimensional tests. *Antimicrobial agents and chemotherapy*, *36*(9), pp.1877-1882.
32. Tzelepi, E., Giakkoupi, P., Sofianou, D., Loukova, V., Kemeroglou, A. and Tsakris, A., 2019. Detection of extended-spectrum β-lactamases in clinical isolates of Enterobacter cloacae and Enterobacter aerogenes. *Journal of clinical microbiology*, *38*(2), pp.542-546.
33. Hattab, S., Ma, A. H., Tariq, Z., Vega Prado, I., Drobish, I., Lee, R., & Yee, R. (2024). Rapid phenotypic and genotypic antimicrobial susceptibility testing approaches for use in the clinical laboratory. *Antibiotics*, *13*(8), 786.
34. Linscott, A.J. and Brown, W.J., 2021. Evaluation of four commercially available extended-spectrum beta-lactamase phenotypic confirmation tests. *Journal of clinical microbiology*, *43*(3), pp.1081-1085.
35. M'Zali, F., 2020. 13. H, Chanawong A, Kerr KG, Birkenhead D, Hawkey PM. Detection of extended-spectrum betalactamases in members of the family enterobacteriaceae: comparison of the MAST DD test, the double disc and the Etest ESBL. *J Antimicrob Chemother*, *45*, pp.881-885.
36. Rahal, J.J., Urban, C. and Segal-Maurer, S., 2022. Nosocomial antibiotic resistance in

multiple gram-negative species: experience at one hospital with squeezing the resistance balloon at multiple sites. *Clinical Infectious Diseases*, *34*(4),pp.499-503.

37. Gupta, K., Hooton, T.M., Naber, K.G., Wullt, B., Colgan, R., Miller, L.G., Moran, G.J., Nicolle, L.E., Raz, R., Schaeffer, A.J. and Soper, D.E., 2020. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clinical infectious diseases*, *52*(5), pp.e103-e120.
38. Davido, B., Crémieux, A.C., Vaugier, I., Gatin, L., Noussair, L., Massias, L., Laurent, F. and Saleh-Mghir, A., 2023. Efficacy of ceftazidime-avibactam in various combinations for the treatment of experimental osteomyelitis due to Klebsiella pneumoniae carbapenemase (KPC)-producing Klebsiella pneumoniae. *International Journal of Antimicrobial Agents*, 61(1), p.106702.
39. Hellmich, B., Sanchez-Alamo, B., Schirmer, J.H., Berti, A., Blockmans, D., Cid, M.C., Holle, J.U., Hollinger, N., Karadag, O., Kronbichler, A. and Little, M.A., 2023. EULAR recommendations for the management of ANCA-associated vasculitis: 2022 update. *Annals of the Rheumatic Diseases*.
40. Tamma, P.D., Aitken, S.L., Bonomo, R.A., Mathers, A.J., Van Duin, D. and Clancy, C.J., 2022. Infectious Diseases Society of America 2022 guidance on the treatment of extended-spectrum β-lactamase producing Enterobacterales (ESBL-E), carbapenem-resistant Enterobacterales (CRE), and Pseudomonas aeruginosa with difficult-to-treat resistance (DTR-P. aeruginosa). *Clinical Infectious Diseases*, *75*(2), pp.187-212.