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Antimicrobial Resistance in Urinary Tract Infections: The Growing Importance of Uropathogens That Produce ESBL

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ABSTRACT

Urinary tract infections (UTIs) are prevalent bacterial illnesses globally and pose a considerable public health concern due to their frequency, recurrence, and growing correlation with antibiotic resistance. Urinary tract infections (UTIs) mostly result from Escherichia coli; however, their management has become more challenging owing to the emergence of Extended-Spectrum Beta-Lactamase (ESBL) manufacturers. Extended-spectrum beta-lactamases (ESBLs) are enzymes that diminish the effectiveness of standard antimicrobial agents by hydrolysing a wide array of β-lactam antibiotics, including penicillins, cephalosporins, and monobactams. The two most common bacteria found to produce ESBL are KlEBsiella pneumonia and Escherichia coli. However, reports of it in other uropathogens such Pseudomonas aeruginosa, Citrobacter freundii, Proteus mirabilis, and Enterobacter cloaca are growing. Due to the presence of resistance determinants for trimethoprim-sulfamethoxazole, aminoglycosides, and fluoroquinolones, these organisms often exhibit multidrug resistance. The taxonomy and mechanisms of extended-spectrum beta-lactamases (ESBLs), the most common uropathogens that produce ESBLs, laboratory detection methods, diagnostic issues, and the epidemiology and risk factors of UTIs are all explained in this paper. Effective infection control, antimicrobial stewardship, and the creation of more effective treatment plans for multidrug-resistant urinary tract infections all depend on an understanding of the transmission and resistance mechanisms of ESBL-producing bacteria.

Keywords: Urinary Tract Infections (UTIs), Extended-Spectrum Beta-Lactamases (ESBLs), Multidrug-Resistant Bacteria, Phenotypic Detection, Whole Genome Sequencing, Uropathogens.

1. INTRODUCTION

Urinary tract infections are a common problem and there are millions of people affected each year in the hospital and community. These could be conditions affecting the urethra, bladder, ureters, kidneys and other parts of the urinary system. UTIs can occur in any age and sex but women are more prone to UTIs due to anatomic and physiologic reasons including the shorter urethra and the proximity of the urethral opening to the anal region. UTI are also common in elderly, pregnant women, ill patients and catheterised patients (1).

Bacterial infections, particularly those brought on by Gram-negative bacteria like Escherichia coli, are the primary cause of

the majority of UTIs. Standard antibiotics such as fluoroquinolones, cephalosporins, and penicillins were easily used to treat infections. However, the overuse and abuse of antibiotics in recent decades have played a major role in the development of antimicrobial resistance in uropathogens (2). The development of multi-drug resistance (MDR) bacteria has made treatment more difficult and raised UTI-related morbidity, death, and medical expenses. emergence of illnesses that are resistant. They pose a serious threat to world health. Among them are microbes that generate ESBLs, or extended spectrum beta-lactamases. Many β-lactam antibiotics, including monobactams and third-generation cephalosporins, may be hydrolysed and rendered inactive by some Gram-negative bacteria that generate enzymes known as ESBLs. Because ESBL-producing bacteria are often resistant to several non-β-lactam antibiotics, infections caused by these bacteria are challenging to cure. Previously only found in hospitals, ESBL-producing bacteria are now more often found in community-acquired illnesses, indicating widespread transmission (3).

2. The prevalence of infections in the urinary tract

Over 150 million people worldwide suffer from urinary tract infections (UTIs), which are among the most common bacterial illnesses. Because of their high frequency, recurrence, and related medical costs, these infections represent a serious public health concern. Urinary tract infections are more prevalent in females than in males, but they may occur at any age, from infancy to old age. Urinary tract infections affect 50 to 60 percent of women at least once in their lives, and many of them repeat. UTIs and antimicrobial resistance are more prevalent in developing countries, primarily due to poor sanitation, limited access to healthcare, and the indiscriminate use of antibiotics (4). Urinary tract infections are also a significant problem in developed countries, especially in hospitalised and catheterised patients. Hospital-acquired UTIs are a major component of nosocomial infections and are often caused by multidrug resistant pathogens. Community-acquired UTIs are usually caused by simple infections, whereas hospital-acquired UTIs are often related to catheterisation, prolonged hospitalisation, invasive procedures and immunosuppression. The global epidemiological picture of UTIs has further been complicated by the increasing frequency of resistant infections, particularly by the ESBL-producing bacteria (5).

3. Risk Factors for UTIs

3.1 Female Gender

Women are also more susceptible to urinary tract infections because of their anatomy. For example, women have a shorter urethra than men, and the urethra is closer to the anus, which makes it easier for bacteria to enter the urinary system. Risk factors include sexual activity, hormonal changes, and use of contraceptives.

3.2 Pregnancy

Hormonal and physiological changes that cause vesicoureteral reflux and urine stasis put pregnant women at higher risk. Pregnancy-related UTIs may cause problems including pyelonephritis, early labour, and l
ow birth weight if they are not treated.

3.3 Diabetes Mellitus

Due to compromised immunological responses, glycosuria, and bladder dysfunction, patients with diabetes mellitus are more vulnerable to UTIs. Additionally, diabetics are more susceptible to infections brought on by ESBL-producing and multidrug-resistant bacteria.

3.4 Catheterization

The most significant risk factor for nosocomial urinary tract infections (UTIs) is urine catheterisation. Bacterial colonisation and biofilm development are facilitated by indwelling catheters, raising the possibility

of antibiotic resistance and chronic infection.

3.5 Immunocompromised Conditions

UTIs and opportunistic infections brought on by resistant organisms are particularly dangerous for those with compromised immune systems, such as HIV/AIDS patients, transplant recipients, and cancer patients receiving chemotherapy (6,7).

4. Classification of UTIs

Table No. 01: Different types of UTIs

S. No.	Type of UTI	Description	Key Characteristics
1	**Uncomplicated UTI**	happens in otherwise healthy individuals with appropriate urinary tract anatomy and function. These infections usually affect the bladder and are easier to manage and treat.	• Healthy individuals • Normal urinary tract structure and function • Usually involves cystitis (bladder infection) • Good response to antibiotic therapy
2	**Complicated UTI**	occurs in people who have immunosuppression, urinary blockage, or anatomical or functional abnormalities of the urinary tract. These infections are often more serious and persistent.	• Associated with urinary tract abnormalities • Common in catheterized patients • Higher risk of recurrence • Frequently caused by multidrug-resistant pathogens
3	**Community-Acquired UTI**	Develops outside healthcare settings and is most commonly caused by *Escherichia coli*. ESBL-producing pathogens have become much more common in community settings in recent years.	• Acquired outside hospitals • Commonly caused by *E. coli* • Increasing occurrence of ESBL-producing strains • Major cause of outpatient infections
4	**Hospital-Acquired (Nosocomial) UTI**	Develops during hospitalization or following medical procedures. These infections are frequently linked to urinary catheterization and exposure to healthcare-associated pathogens.	• Occurs ≥48 hours after hospitalization • Strongly associated with catheter use • Often caused by multidrug-resistant organisms • Includes ESBL-producing bacteria and other resistant pathogens (8,9)

5. Major Uropathogens Responsible for UTIs

Although a variety of microorganisms may cause UTIs, bacteria are the most common etiological culprits.

Table No. 02: Various major Uropathogens

Uropathogen	Major Virulence Factors	Clinical Relevance in UTIs
Proteus mirabilis	Urease, swarming motility, biofilm formation	Complicated UTIs, catheter-associated infections, urinary stone formation
Enterobacter cloacae	Biofilm formation, environmental	Nosocomial and multidrug-resistant UTIs

	persistence	
Citrobacter freundii	Environmental survival, opportunistic pathogenicity	UTIs in elderly and immunocompromised patients
KlEBsiella oxytoca	Capsule formation, adhesins, biofilm production	Community- and hospital-acquired UTIs
Morganella morganii	Urease production, motility	Opportunistic UTIs, especially in hospitalized patients
Serratia marcescens	Biofilm formation, environmental persistence	Catheter-associated and healthcare-associated UTIs
Providencia stuartii / *P. rettgeri*	Urease production, biofilm formation	Chronic catheter-associated UTIs
Pseudomonas aeruginosa	Biofilm formation, efflux pumps, low membrane permeability	Severe complicated and multidrug-resistant UTIs (10,11)

6. Extended Spectrum Beta-Lactamases (ESBLs)

Extended spectrum beta-lactamases (ESBLs) are a broad group of enzymes produced by gram-negative bacteria that confer resistance to monobactams such as aztreonam, first, second, and third generation cephalosporins, and penicillins. These enzymes hydrolyse the β-lactam ring, which is essential for the antibacterial effect of many drugs, rendering them inactive. ESBL producers are often associated with members of the Enterobacterales family, which includes important UTI-causing bacteria including Escherichia coli and KlEBsiella pneumoniae. One characteristic of ESBLs is that their activity is often suppressed by β-lactamase inhibitors such tazobactam, sulbactam, and clavulanic acid (12). The emergence of ESBL-producing bacteria is a serious public health problem since there are few therapies available and treatment failure rates associated with these infections are increasing. The history of ESBLs began in the early 1980s when bacterial isolates resistant to newly created third-generation cephalosporins were discovered in Europe. Point mutations of previously known narrow-spectrum β-lactamases gave rise to these enzymes, which have the ability to hydrolyse a greater variety of β-lactam antibiotics. ESBLs have rapidly spread across hospitals and communities throughout the world since their discovery (13).

7. Types of ESBL Enzymes

7.1 TEM-Type ESBLs

One of the first enzymes to be identified as ESBLs were TEM-type β-lactamases, which are still a significant group of resistance factors. The first strain of E. coli that produced the TEM-1 enzyme was obtained in Greece in the 1960s from a patient named "Temoniera," which is where the term TEM originates. At first, the enzymes TEM-1 and TEM-2 were limited to hydrolysing penicillins and early cephalosporins. Nevertheless, several mutations have accumulated in the genes encoding these enzymes due to the selection pressure of the extensive usage of extended range cephalosporins. Cefotaxime, ceftriaxone, and ceftazidime are examples of third-generation cephalosporins that the enzyme may hydrolyse thanks to these mutations that changed its active site. There are more than 200 known TEM variations, some of which exhibit activity throughout a wider range. TEM-type ESBLs are often found in K. pneumoniae, E. coli, and other Enterobacterales, and they were a key factor in the first global spread of ESBL-mediated resistance (14).

7.2 SHV-Type ESBLs

The SHV (Sulfhydryl Variable) family of β-lactamases is a major group of ESBL enzymes. SHV-1 is an intrinsic property of K. pneumonia and was initially associated with resistance to ampicillin. Mutations in SHV genes yielded variants with extended spectrum cephalosporin hydrolytic activity, similar to TEM enzymes. ESBLs derived from SHV became more prevalent in hospital settings in the 1980s and 1990s and

were a major cause of outbreaks of multidrug-resistant KlEBsiella infections. Various SHV variants have been described: SHV-2, SHV-5, SHV-12, etc. They differ in their hydrolytic profiles and geographic distribution (15).

7.3 CTX-M-Type ESBLs (Currently the Most Prevalent)

The CTX-M enzymes belong to the most common and therapeutically important family of extended-spectrum beta-lactamases worldwide. The term CTX-M originates from their significant hydrolytic activity against cefotaxime ("CTX") and Munich ("M"), where some of the earliest versions were found. Unlike TEM and SHV enzymes, CTX-M β-lactamases are derived from chromosomal β-lactamase genes of environmental Kluyvera species and have been mobilised to plasmids. Since the 1990s, CTX-M enzymes have proliferated in both clinical and community settings, making them the most prevalent kind of extended-spectrum beta-lactamase (ESBL) worldwide. The widespread use of cephalosporins in human and animal health, the presence of CTX-M enzymes on highly mobile plasmids, and their association with viable bacterial clones all contribute to the enzymes' global spread. CTX-M-producing bacteria have been a major target of research to identify antibiotic resistance because of their ubiquitous presence (16).

7.4 Genetic Basis of ESBL Production

7.4.1 Plasmid-Mediated Genes

Genes encoding ESBLs are largely located on plasmids, which are small, extrachromosomal DNA structures that self-replicate in bacterial cells. Plasmids play a major role in the spread of antimicrobial resistance because they can carry many resistance genes simultaneously and facilitate their transfer among bacteria. Common ESBL genes are blaTEM, blaSHV and blaCTX-M that encode the respective β-lactamase enzymes. These plasmids frequently also carry additional resistance determinants towards aminoglycosides, fluoroquinolones, sulfonamides and tetracyclines, resulting in multidrug resistant bacterial strains. The plasmid-mediated nature of ESBL genes allows rapid spread among and between species of bacteria and between geographical areas, thereby greatly complicating the global problem of antibiotic resistance (17).

7.4.2 Horizontal Gene Transfer

One of the main ways that ESBL genes spread among bacterial populations is by horizontal gene transfer (HGT). Unlike vertical gene transfer, which is the transfer of genetic material from parent to child cells during replication, horizontal gene transfer allows genetic material to flow across unrelated microbes. The primary method of ESBL spread is conjugation, which involves the direct transfer of plasmids containing resistance genes from one bacterial cell to another via specialised structures known as pili. Although they are less common in Enterobacterales, transformation and transduction may also aid in the transmission of resistance genes (18).

7.5 Role of Integrons and Transposons

Mobile genetic elements called integrons and transposons are crucial for the acquisition, accumulation, and spread of ESBL genes. Integrons are genetic elements that use site-specific recombination processes to capture and express resistance gene cassettes. They facilitate the accumulation of many resistance genes inside a single bacterial cell, hence fostering multidrug resistance. DNA sequences that may travel across the genome or between plasmids and chromosomes are known as transposons, or "jumping genes." The transposons are mobile and transferable to other bacterial hosts due to the many ESBL genes that are inserted into them (19).

8. Mechanisms of Antibiotic Resistance Associated with ESBLs

8.1 Hydrolysis of beta-Lactam Antibiotics

These enzymes are known as Extended Spectrum Beta-Lactamases (ESBLs) and

mediate resistance primarily by hydrolysing the β-lactam ring of β-lactam antibiotics. Typically, beta-lactam antibiotics attach to enzymes known as penicillin-binding proteins (PBPs), which are needed to build the bacterial cell wall. This interaction blocks peptidoglycan cross-linking, destabilising the cell wall and killing the bacterium. ESBLs are enzymes that reside in the periplasmic region of gram-negative bacteria and recognise and bind the β-lactam ring of susceptible compounds. ESBLs catalyse the cleavage of the amide bond of the β-lactam ring, converting the active antibiotic into an inactive metabolite, through a serine-dependent mechanism. Hydrolysis of the antibiotic leads to suppression of cell wall synthesis and loss of affinity for penicillin-binding proteins (PBPs).

8.2 Resistance to Penicillins

The antibacterial action of penicillins depends on the presence of a special β-lactam ring. Once penicillin molecules penetrate the bacterial periplasmic area, ESBL enzymes identify them. The β-lactam ring's carbonyl carbon is attacked by the serine residue in the enzyme's active site, creating an acyl-enzyme intermediate. An inactive antibiotic molecule is released when the ring structure is broken by the ensuing hydrolysis. Penicillins cannot attach to PBPs due to the breakdown of the β-lactam ring, which permits bacterial growth and peptidoglycan formation. ESBL-producing bacteria may effectively inactivate a number of penicillin derivatives via the enzymatic degradation process.

8.3 Cephalosporins Resistance

Cephalosporins are like penicillins in that they have a β-lactam ring, but they have been chemically altered to increase antibacterial efficacy and stability. ESBL enzymes have evolved to bind and hydrolyse extended-spectrum cephalosporins as a consequence of amino acid changes that enlarge and alter the structure of their active site. ESBL enzymes interact with the antibiotic molecule and help the β-lactam ring to break down when exposed to cephalosporins. This hydrolysis removes the affinity of the antibiotic for PBPs, and prevents suppression of cell wall production. CTX-M-type extended-spectrum beta-lactamases (ESBLs) are especially good at hydrolysing cefotaxime and ceftriaxone. TEM- and SHV-derived ESBLs are able to hydrolyse a wide spectrum of cephalosporins. Enzymatic degradation of cephalosporins significantly diminishes their antibacterial activity and promotes bacterial persistence under antibiotic pressure.

8.4 Resistance to Monobactams

Penicillin binding proteins (PBPs) involved in the formation of bacterial cell walls are bound by monobactams, which contain a monocyclic β-lactam ring. One excellent example of a monobactam is aztreonam. Monobactams were originally designed to resist hydrolysis by a variety of classic β-lactamases, but ESBL enzymes have evolved to recognise and hydrolyse these molecules. During the periplasmic phase, ESBL enzymes bind to the β-lactam ring of aztreonam and promote ring opening via hydrolysis (20,21).

8.5 Co-Resistance to Other Antibiotics

The resistance of ESBL-producing bacteria to several non-β-lactam antibiotic classes at the same time is known as co-resistance. The problem is mainly due to ESBL genes being frequently situated on transferable plasmids which also bear resistance determinants for other antimicrobial agents. Acquisition of a single, unique resistant plasmid could confer multi-drug resistance through a variety of biological mechanisms.

8.5.1 Fluoroquinolone co-resistance

Chromosome alterations and plasmid-mediated quinolone resistance genes are often responsible for fluoroquinolone resistance in ESBL-producing bacteria. The qnr genes are plasmid-mediated and produce proteins that bind to and shield

topoisomerase IV and DNA gyrase, preventing fluoroquinolone drugs from effectively interacting with these target enzymes. Other plasmid-encoded genes, such as aac (6')-Ib-cr, encode specific altered acetyltransferases that chemically modify certain fluoroquinolones, thereby reducing their antibacterial activity. Moreover, efflux pump genes such as qepA promote the active expulsion of fluoroquinolones from bacterial cells, thus reducing intracellular drug concentrations. Mutations in the genes encoding DNA gyrase and topoisomerase IV reduce antibiotic affinity and promote resistance.

8.5.2 Cross-resistance with aminoglycosides

In ESBL-producing bacteria, aminoglycoside resistance is primarily mediated by aminoglycoside-modifying enzymes encoded by plasmid-associated resistance genes. These enzymes chemically modify aminoglycosides by acetylation, phosphorylation or adenylation. Aminoglycosides may be acetylated by acetyltransferases (AACs), phosphorylated by phosphotransferases (APHs) or adenylated by nucleotidyltransferases (ANTs). These alterations prevent aminoglycosides from binding correctly to the bacterial 30S ribosomal subunit and thus eliminate their ability to inhibit protein synthesis. Other mechanisms include decreased permeability of the outer membrane and increased activity of efflux pumps, both of which further reduce the intracellular concentration of antibiotics.

8.5.3 Co-Resistance to Trimethoprim-Sulfamethoxazole

Plasmid-encoded genes encoding altered target enzymes of folate biosynthesis promote resistance to trimethoprim-sulfamethoxazole (TMP-SMX). Resistance genes for sulfonamides (sul1, sul2 and sul3) encode modified forms of dihydropteroate synthase (DHPS) with decreased affinity for sulfamethoxazole. Similarly, trimethoprim resistance genes (dfr genes) encode alternative dihydrofolate reductase (DHFR) enzymes that are not sufficiently inhibited by trimethoprim. These modified enzymes allow continued folate synthesis even in the presence of TMP-SMX. This enables the bacteria to survive despite the antibiotic intervention. By allowing the folate metabolism to continue (important for nucleic acid synthesis and bacterial proliferation) (22,23).

9. ESBL-producing uropathogens in urinary tract infections

Table No. 03: Basic ESBL-Producing Uropathogens Linked to Urinary Tract Infections (24,25)

S. No.	Organism	Major ESBL Enzymes	Clinical Significance
1	Escherichia coli	CTX-M (especially CTX-M-15), TEM, SHV	Most common cause of community- and hospital-acquired UTIs; exhibits resistance to penicillins, third-generation cephalosporins, and monobactams, limiting treatment options.
2	Klebsiella pneumoniae	CTX-M, SHV	Major cause of healthcare-associated and catheter-associated UTIs, pyelonephritis, and urosepsis; frequently multidrug resistant.
3	Proteus mirabilis	CTX-M, TEM, SHV	Associated with complicated and catheter-associated UTIs; contributes to urinary stone formation and chronic infections.
4	Enterobacter cloacae	CTX-M, SHV, TEM	Important nosocomial pathogen causing complicated UTIs; often multidrug resistant and difficult to treat.
5	Citrobacter freundii	CTX-M, SHV	Causes opportunistic UTIs, particularly in elderly and immunocompromised patients; serves as a reservoir for antimicrobial resistance genes.

9.1 Other gram-negative bacteria producing ESBL

In addition to Escherichia coli and KlEBsiella pneumoniae, other Gram-negative bacteria have emerged as important ESBL-producing uropathogens causing urinary tract infections (UTIs). The organisms *are Proteus mirabilis, Enterobacter cloacae, Citrobacter freundii, KlEBsiella oxytoca, Morganella morganii, Serratia marcescens, Providencia stuartii, Providencia rettgeri* and *Pseudomonas aeruginosa*. Although they represent a lower proportion of UTIs than E. coli and K. Together with P. pneumoniae, these organisms have become very important clinically by acquiring and spreading ESBL genes, especially belonging to the families of CTX-M, TEM and SHV (26).

Table No. 04: Other Common ESBL Producing Gram Negative Uropathogens

S. No.	Category	Major Organisms	Clinical Significance
1.	Gram-Negative Bacteria	*E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*	Responsible for the majority of UTIs and commonly associated with antimicrobial resistance
2.	Gram-Positive Bacteria	*E. faecalis*, *S. saprophyticus*	Important causes of nosocomial and uncomplicated UTIs
3.	Emerging MDR Uropathogens	ESBL-producing *E. coli*, *K. pneumoniae*, and other Enterobacteriaceae	Major public health concern due to extensive antimicrobial resistance and treatment failure

10. Detection of ESBL producing bacteria in Laboratory

10.1 Phenotypic Methods for the Detection of ESBL-producing Enterobacteriaceae

Phenotypic methods are still the most common laboratory methods for the identification of Extended-Spectrum β-Lactamase (ESBL)-producing bacteria. These methods exploit the ability of β-lactamase inhibitors, such as clavulanic acid, to inhibit ESBL enzymes and restore the efficacy of β-lactam antibiotics. Phenotypic assays are cost-effective, easy to perform and are adaptable to routine clinical microbiology laboratories. The main phenotypic methods employed are double disc synergy test (DDST), combination disc test (CDT) and E-test method.

10.1.1 DDST (Double Disc Synergy Test)

One of the first and most widely used screening techniques for identifying ESBL-producing bacteria is the Double Disc Synergy Test (DDST). The synergistic interaction between a β-lactam antibiotic and a β-lactamase inhibitor is the basis for this test. On a Mueller-Hinton agar plate, a suspension of 0.5 McFarland standards of the bacteria is equally distributed. Third-generation cephalosporin discs, such as cefotaxime, ceftazidime, ceftriaxone, or aztreonam, are positioned at a consistent distance (often 20–30 mm centre to centre) from the amoxicillin-clavulanic acid disc in the centre of the plate. After that, the plate is incubated for 18–24 hours at 35–37 °C. Following incubation, the generation of

ESBL is indicated by an expanded inhibition zone or a clear "keyhole" appearance between the cephalosporin disc and the clavulanic acid disc. This action is explained by clavulanic acid's suppression of the ESBL enzyme, which restores the antibiotic's antibacterial activity. The DDST is easy to use, reasonably priced, and appropriate for screening a lot of clinical isolates. However, in organisms that generate significant quantities of AmpC b-lactamases or other resistance mechanisms, the test may not identify ESBLs well, leading to false-negative findings. The distance between antibiotic discs and the bacterial isolate's degree of enzyme expression have an impact on DDST accuracy.

10.1.2 Test for Combination Disc (CDT)

Combination Disc Test (CDT) is a validated phenotypic method recommended by international organisations, such as Clinical and Laboratory Standards Institute (CLSI) for identification of ESBL. The test measures the inhibitory effect of a cephalosporin antibiotic alone, compared with its effect in combination with clavulanic acid. The most used combinations are ceftazidime + ceftazidime-clavulanic acid and cefotaxime + cefotaxime-clavulanic acid.

This is done by applying a standardised bacterial inoculum to a Mueller–Hinton agar plate. Discs containing only cephalosporin and discs containing cephalosporin and clavulanic acid are placed on the surface of the agar at an appropriate distance from each other. The inhibition zones around the discs are measured after incubation at 35–37oC for 18–24 hours. The combination Disc Test is more sensitive and specific and thus more reliable than DDST. It generates unambiguous and clear results and is easy to interpret. This method is simple, consistent and correctly detects ESBL producing isolates; thus, it is commonly used in clinical laboratories. However, as with other phenotypic methods, its performance may be influenced by the simultaneous presence of AmpC β-lactamases, carbapenemases or porin mutations.

10.1.3 E-test Procedure

ESBL-producing bacteria may be identified and confirmed using a quantitative phenotypic test called the E-test technique. It makes use of a plastic strip with a continuous gradient of a cephalosporin antibiotic on one end and the same antibiotic mixed with clavulanic acid on the other. Cefotaxime/cefotaxime-clavulanic acid and ceftazidime/ceftazidime-clavulanic acid combos are common E-test strip combinations. The E-test strip is placed on the surface of a Mueller-Hinton agar plate inoculated with a uniform bacterial solution. An elliptical zone of inhibition develops around the strip during 18–24 hours of incubation at 35–37°C. The scale shown on the strip is used to determine the minimum inhibitory concentration (MIC) values. If the MIC of the antibiotic tested in the presence of clavulanic acid is at least eight times lower (≥3 two-fold dilutions) than the antibiotic alone, or if the inhibition ellipse clearly changes, ESBL formation is verified. The E-test is very sensitive and provides a qualitative and quantitative indication of antimicrobial susceptibility. It is especially useful for detecting ESBLs in isolates with borderline resistance and for determining accurate MICs that can guide antibiotic therapy. The E-test has advantages over DDST and CDT, but it is more expensive and cannot be used routinely in resource-poor laboratories. However, it is considered as an important confirmatory technique in research and reference laboratories because of its high accuracy and simple interpretation (27).

10.2 Molecular Methods for Detection of ESBL Producers

Extended-Spectrum β-Lactamase (ESBL)-producing bacteria must now be accurately identified and characterised using molecular detection methods. While phenotypic approaches may detect the functional activity of ESBL enzymes, molecular

methods can determine the genetic determinants of beta-lactam resistance. These methods have improved repeatability, sensitivity, and specificity—all of which are crucial for epidemiological research, surveillance studies, epidemic monitoring, and the clarification of the propagation of antibiotic resistance genes. The two main molecular methods for identifying infections that produce ESBL are PCR and WGS.

10.2.1 Detection of ESBL Genes by PCR

Polymerase Chain Reaction (PCR) is the main method used to identify ESBL genes in clinical bacterial isolates at the molecular level. It is based on the amplification of specific sequences of DNA corresponding to genes for the expression of ESBL enzymes. PCR is an essential tool in the clinical microbiology laboratory and in research settings. It allows for rapid and precise identification of resistance genes, often within a matter of hours. The process begins with the isolation of genomic DNA from bacterial isolates from clinical samples such as urine, blood, sputum or wound swabs. The isolated DNA is then used as a template for amplification with primers specifically designed to target certain ESBL genes. The most commonly detected ESBL genes are from the blaTEM, blaSHV and blaCTX-M families, but other genes such as blaOXA, blaPER, blaVEB and blaGES may also be present, depending on the bacterial species and the geographic location.

Repeated cycles of denaturation, primer annealing and DNA extension during PCR amplification result in exponential amplification of target gene sequences. The amplified products are then analysed by agarose gel electrophoresis, and DNA bands of the expected sizes indicate the presence of certain ESBL genes. A modification of PCR, multiplex PCR, allows simultaneous detection of multiple ESBL genes in one reaction, thus increasing efficiency and reducing laboratory costs. There are many benefits of PCR over the phenotypic methods. It allows for rapid and precise identification of resistant genes even in isolates with deviant behavioural characteristics.

10.2.2. Whole Genome Sequencing (WGS)

Whole Genome Sequencing (WGS) is the most comprehensive molecular technique for identifying ESBL-producing bacteria and exploring the genetic basis of antibiotic resistance. The whole nucleotide sequence of a bacterial genome is provided by whole genome sequencing (WGS), which enables a thorough analysis of all resistance genes, virulence factors, plasmids, mobile genetic elements, and phylogenetic connections in a single experiment. The WGS methodology begins with the extraction of high-quality bacterial DNA, followed by library preparation and sequencing on state-of-the-art platforms (e.g., Illumina, Oxford Nanopore Technologies or Pacific Biosciences systems). The bacterial genome is reconstructed by merging many small and large pieces of DNA with bioinformatics techniques. The genomic data is then compared with specialised antibiotic resistance databases to identify ESBL genes and other resistance determinants. In addition to identification of classical ESBL genes (blaCTX-M, blaTEM, blaSHV), WGS can also identify genes encoding carbapenemases, AmpC β-lactamases, aminoglycoside-modifying enzymes, quinolone resistance determinants, and a number of other antimicrobial resistance mechanisms in parallel. This study is detailed and provides a comprehensive resistance profile of the bacterial isolate.

This approach has provided important insights into the emergence of high-risk international clones, e.g. E. coli ST131 associated with CTX-M-mediated ESBL production and global recurrent urinary tract infections. However, there are limitations to WGS, despite its many advantages. The method needs special sequencing machines, good bioinformatics skills and a lot of computer power. Moreover, it is also more expensive and time-consuming than

traditional PCR, which restricts its normal application in many clinical laboratories (28).

11. Problems in ESBL detection in clinical laboratories

Sensitivity, specificity and reliability of ESBL detection methods can be affected by several clinical laboratory problems. The challenges are due to the heterogeneity of the ESBL enzymes, the diversity of resistance mechanisms, the technological limitations of the diagnostic methods and the resource constraints. The main difficulties are described below.

11.1 Simultaneous Presence of Multiple β-Lactamases

A major problem in ESBL detection is the presence of ESBLs in combination with other β-lactamases, including AmpC β-lactamases and carbapenemases. Many Gram-negative bacteria synthesise several β-lactamase variants simultaneously, complicating phenotypic interpretation. Clavulanic acid, frequently used in ESBL detection assays, does not inhibit AmpC enzymes. Inhibitory action of clavulanic acid may be masked, leading to false-negative results. The presence of carbapenemases can affect the production and detection of ESBL activity. The convergence of resistance mechanisms makes laboratory diagnosis difficult and may contribute to under-reporting of ESBL-producing organisms.

11.2 Variation in ESBL Gene Expression

The expression levels of the ESBL genes in various bacterial isolates are determined by the genetic factors, the number of copies of the plasmid, the efficiency of the promoter and environmental conditions. ESBLs are produced in small amounts by some bacteria, leading to subtle patterns of phenotypic resistance that may be missed by routine screening techniques. In contrast, high expression may give rise to unusual susceptibility patterns. This heterogeneity can lead to inconsistent test results and difficulties in distinguishing ESBL producers from non-producers, particularly when only phenotypic approaches are employed.

11.3 Limitations of Phenotypic Detection Techniques

Despite their widespread usage, phenotypic techniques like the Double Disc Synergy Test (DDST), Combination Disc Test (CDT), and E-test have drawbacks. Results may be affected by factors such as disc placement, inoculum density, incubation conditions and criteria for assessment. Some of the ESBL producers may not demonstrate clear synergy pattern especially when resistance is low. Moreover, some ESBL mutations may not be detected by standard phenotypic testing. Such limitations can reduce diagnostic accuracy and require confirmatory molecular testing.

11.4 New variants of ESBL

The continuous evolution of ESBL genes creates a major challenge for laboratory identification. Mutations and horizontal gene transfer give rise to novel ESBL variants that differ in their resistance and substrate profiles. Hence, new emerging or rare ESBL variants may go undetected in the absence of specific primers. This could jeopardise monitoring efforts and slow down the rapid identification of growing resistance problems.

11.5 Differentiating ESBLs from Other Resistance Mechanisms

Mechanisms other than ESBL formation could be responsible for resistance to third-generation cephalosporins, such as AmpC overproduction, porin loss, efflux pump activation and carbapenemases production. These methods can give susceptibility patterns comparable to those of ESBL-producing bacteria. However, standard phenotypic assays alone often fail to distinguish between these resistance pathways. Such a misinterpretation might lead to misclassification of isolates and to wrong choices of treatment (29).

12. CONCLUSION

Due to their high frequency, recurrence, and growing correlation with antibiotic resistance, urinary tract infections represent a significant worldwide health concern. The occurrence and swift spread of ESBL-producing bacteria have greatly affected the treatment and management of UTIs in community and clinical environments. ESBL-producing pathogens, mainly Escherichia coli and KlEBsiella pneumoniae, have acquired the ability to hydrolyse an extended spectrum of β-lactam antibiotics resulting in reduced therapeutic efficacy and increased treatment failures. Widespread dissemination of ESBL genes through plasmids, integrons, transposons and horizontal gene transfer has rapidly disseminated multidrug-resistant uropathogens worldwide.

Accurately identifying ESBL-producing bacteria in the laboratory is fundamental to appropriate antimicrobial therapy and management of disease. While molecular techniques like PCR and whole genome sequencing may provide a thorough knowledge of the mechanisms of resistance and epidemiological trends, phenotypic approaches are feasible and affordable for routine diagnosis. In conclusion, the management of ESBL-associated UTIs requires a comprehensive strategy that includes prompt diagnosis, rational drug use, antimicrobial stewardship programs, infection control measures, and continuous monitoring of resistance patterns. Future research should be directed to the development of novel antimicrobial agents, rapid diagnostic methods and alternative therapeutic approaches to address the increasing threat of multidrug resistant uropathogens and improve patient outcomes worldwide.

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