GENETIC METHODS FOR DETECTION OF ANTIBIOTIC RESISTANCE: FOCUS ON EXTENDED-SPECTRUM β-LACTAMASES

Magdalena Chroma*, Milan Kolar

Department of Microbiology, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic
E-mail: magdalena.chroma@centrum.cz

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Background. In 1928, the first antibiotic, penicillin, was discovered. That was the beginning of a great era in the development and prescription of antibiotics. However, the introduction of these antimicrobial agents into clinical practice was accompanied by the problem of antibiotic resistance. Currently, bacterial resistance to antibiotics poses a major problem in both hospital and community settings throughout the world.

Methods and results. This review provides examples of modern genetic methods and their practical application in the field of extended-spectrum β-lactamase detection. Since extended-spectrum β-lactamases are the main mechanism of Gram-negative bacterial resistance to oxyimino-cephalosporins, rapid and accurate detection is requested in common clinical practice.

Conclusions. Currently, the detection of extended-spectrum β-lactamases is primarily based on the determination of bacterial phenotypes rather than genotypes. This is because therapeutic decisions are based on assessing the susceptibility rather than presence of resistance genes. One of the main disadvantages of genetic methods is high costs, including those of laboratory equipment. On the other hand, if these modern methods are introduced into diagnostics, they often help in rapid and accurate detection of certain microorganisms or their resistance and pathogenic determinants.

INTRODUCTION

The first antibiotic, penicillin, was discovered in 1928 by Sir Alexander Fleming, a Scottish biologist and pharmacologist. However, it took several years before penicillin could be isolated and used as a medicine. This discovery is attributed to a German biochemist Ernst Chain and an Australian pharmacologist Howard Florey, who successfully isolated penicillin in 1939 and used it to treat bacterial infection during World War II. That was the beginning of a great era in the discovery, development and prescription of antibiotics1. Many bacterial infectious diseases causing death in humans were brought under control by the expanded use of these antimicrobial agents but only a few years after introducing penicillin into clinical practice, penicillin-resistant strains were reported2,3. Currently, bacterial resistance to antibiotics represents a major clinical problem all over the world, potentially leading to treatment failure or even patient death where resistant bacteria are etiological agents of serious infections.

Determination of susceptibility or resistance using classical phenotypic tests is the gold standard in clinical microbiology4. However, modern genetic methods, mainly used in university hospitals as a part of medical research or in reference laboratories, are a potentially rapid tool for improving the detection of resistant strains and could contribute to the rational prescription of antibiotics.

A large number of interesting reviews on molecular detection of antimicrobial resistance have been published5-7. These describe the principles and practical applications of the most frequently reported genetic methods for assessing the presence of resistance genes or detection of mutations associated with antimicrobial resistance. The aim of this review is to discuss examples where genetic techniques have been used to characterize extended-spectrum β-lactamases and their genetic nature as an important mechanism of Gram-negative bacterial resistance to oxyimino-cephalosporins.

BACTERIAL RESISTANCE TO ANTIBIOTICS

Antibiotic resistance has developed as a natural evolutionary response of bacteria following their exposure to these drugs and is defined as the ability of bacteria to withstand the inhibition concentration of an antibiotic. It can be intrinsic (naturally occurring) or acquired. The first type is defined as resistance characteristic for all members of a given bacterial species or genus. It results from an inability of the antibiotic to reach its target, a lack of affinity for the target, the presence of efflux pumps or possession of other chromosomal resistance mechanisms8. However, from the clinical aspect, acquired resistance is much more important and significant because of the possible spread of resistant genes through a sensitive microbial population. These genes can be collected on a variety of gene transfer systems, such as bacterial conjugative plasmids, transposable elements and integron systems, that move the genes responsible for antibiotic resistance from one DNA system to another and from a resistant bacterial cell to a sensitive one9.
MECHANISM OF ANTIBIOTIC RESISTANCE AND THEIR GENETIC BASIS

There are many mechanisms that bacteria exhibit to protect themselves from antibiotics. These can be classified into four basic groups:

1. Target site alteration
   Alteration in the primary site of action can arise from mutations at the target gene resulting in a modified target structure. This site still retains its essential cellular function but is inaccessible by antibiotic inhibition. Rifampicin resistance in *Mycobacterium tuberculosis* has arisen due to mutations in *rpoB*, encoding a β-subunit of RNA polymerase, a target site for rifampicin10,11. Mutations in the 16S rRNA gene, for example, confer resistance to the aminoglycosides11,12. Another type of alteration is the importation of a gene specifying a new replacement enzyme that has markedly decreased sensitivity to the drug. The best known example of this mechanism is the acquisition and expression of the * mecA* genes encoding methicillin resistance in *Staphylococcus aureus* resulting in production of altered PBP2a proteins with reduced sensitivity for β-lactam antibiotics13.

2. Change in membrane permeability
   Bacterial cells have an intrinsic capacity to restrict the entry of small molecules. Such ability is typical for Gram-negative bacteria whose outer membrane, an asymmetric bilayer composed of phospholipids, polysaccharides and proteins, provides an effective barrier and first-line defense against antimicrobial agents14. Some proteins form water-filled channels called porins that permit the diffusion of hydrophilic solutes to the cell. Small antibiotics such as β-lactams, tetracycline, chloramphenicol and fluoroquinolones with hydrophilic character also utilize this pathway to cross the outer membrane15. Any decrease in the ability or rate of entry of these compounds can lead to resistance in many bacteria through the loss of functional porins16. Outer membrane porin analysis has revealed that cefoxitin and ceftazidime resistance can be mediated by reduced permeability of Omp K35 and OmpK36 porins in the isolates of *Klebsiella pneumoniae* and *Escherichia coli*17. Deficiency of the outer-membrane protein OprD is one of the basic mechanisms of resistance to imipenem in *Pseudomonas aeruginosa*18,19.

3. Antibiotic efflux
   Efflux pumps are transmembrane transport proteins, used physiologically in Gram-positive and Gram-negative bacteria for exporting specific metabolites and xenobiotic toxic substances out of the cell. As an energy source they utilize the proton motive force13. Pumps may be specific for one substrate or may transport a range of structurally different compounds (including multiple antibiotics); such transport proteins can be associated with multiple drug resistance20. Tetracycline pumps are probably the best studied efflux system in both Gram-positive and Gram-negative bacteria. When tetracycline enters a cell, it is bound with high affinity to the TetR protein that functions as a repressor of the Tet pump gene tetA in *E. coli*, resulting in overproduction of the 42-kDa TetA pump. This transport protein inserts subsequently into the cytoplasmic membrane and acts in antiport mode with entering protons to pump out tetracycline13. Resistance to carbapenems in *P. aeruginosa* can arise from overexpression of the MexAB-OprM efflux system, which contributes to the intrinsic resistance of these bacteria to most β-lactams and many other structurally unrelated antimicrobial agents by exporting them out of the cell21,22.

4. Enzymatic inactivation or destruction of the drug
   Bacterial β-lactamases are probably the most well-known example of this resistance mechanism. These enzymes may have evolved from β-lactam-binding enzymes because of the high relatedness to the penicillin-binding proteins (PBP’s)23. The effect of this group of enzymes consists of the interaction with the β-lactam antibiotic and subsequent disruption of the amide bond in the four-membered β-lactam ring, rendering the antibiotic inactive24. According to the Bush-Jacoby-Medeiros classification scheme, based on the substrate specificity and sensitivity to inhibitors, β-lactamases can be divided into 4 groups (1-4)25. The most widely used Ambler molecular classification scheme of β-lactamases is, on the other hand, based on the nucleotide and amino acid sequences of these enzymes. To date, four classes are recognized (A-D)25. β-lactamases are widespread among many bacterial species of Gram-positive or Gram-negative bacteria. The genes encoding production of these enzymes can be located on the bacterial chromosome or on mobile genetic elements like plasmids and transposons or can occur as a part of integrons located in these transferable elements.

In Gram-negative bacteria, β-lactamase production remains the most important mechanism of resistance to β-lactam antibiotics. One group of these enzymes called extended-spectrum β-lactamases (ESBLs) is a serious clinical problem resulting from possible treatment failure when oxyimino-cephalosporins are used27. ESBLs, belonging to Ambler molecular class A (partially class D) and to class 2be according to the Bush-Jacoby-Medeiros classification scheme, are β-lactamases capable of hydrolyzing oxyimino-cephalosporins (e.g. cefotaxime, ceftazidime, ceftiraxone) and monobactams. They are not active against cephamycins and carbapenems. Generally, they are inhibited by β-lactamase inhibitors such as clavulanate and tazobactam28. Most ESBLs are derivatives of TEM and SHV enzymes. They have arisen through specific point mutations inside the gene of their parent enzymes (TEM-1, TEM-2, SHV-1). These plasmid-mediated β-lactamases are often the cause for resistance to newer cephalosporins and monobactams in members of the family *Enterobacteriaceae*29,30. The CTX-M enzyme group preferentially hydrolyze cefotaxime rather than ceftazidime and are better inhibited by tazobactam than by sulbactam24. ESBL-type members of the OXA-family (OXA-18 and derivatives of OXA-2 and OXA-10) are rare in *Enterobacteriaceae*, but are mostly found in *P. aeruginosa*11. Carbapenemases are relatively new but rapid spreading group of β-lactamases. They confer resistance to the
carbapenems (e.g. imipenem, meropenem, ertapenem) as well as extended spectrum cephalosporins. According to the Ambler classification scheme, carbapenemases fall into class A (KPC, SME, NMC-A, IMI, GES), class B (IMP, VIM) and D (OXA enzymes).32.

GENETIC DETECTION OF ANTIMICROBIAL RESISTANCE

**Genetic techniques used for detection of extended-spectrum β-lactamases**

Since its development in 1985, polymerase chain reaction (PCR) as a basic genetic method has become a powerful detection tool in many scientific areas. In medical microbiology, PCR is not only a technique for detection and identification of important human pathogens but it is also used to detect properties of these microorganisms, such as antimicrobial resistance and virulence factors. The determination of antimicrobial susceptibility of a clinical pathogen indicated as an important etiological agent is crucial for adequate antimicrobial therapy. However, the presence of a resistance gene does not necessarily lead to treatment failure. Various genetic techniques based on either amplification of the target nucleic acid or applications of specific probes have been developed for detection of antimicrobial resistance genes and their expression.

**DNA probes and oligotyping**. Early detection of β-lactamase genes was performed using DNA probes. Hybridization probes labeled with 32P were used for identification of TEM-1, SHV-1, OXA-1 and OXA-2 (the ancestors of ESBLs) in 122 clinical isolates. Although some cross-hybridization was found, only six strains gave false-positive reactions, and the procedure was 99% specific.34 Ouellette et al.35 constructed specific TEM-1 and OXA-1 oligonucleotide probes terminally labeled with T4 polynucleotide kinase and [γ-32P]ATP and found 96–100% correlation in identification of relevant β-lactamase genes compared with the isoelectric focusing technique.

The first ESBLs studies were focused on TEM vari-
single-stranded DNA in non-denaturing polyacrylamide gel is very sensitive to primary sequence. Any difference in the base sequence of an ssDNA sample, due to a mutation or polymorphism, will be detected as a mobility shift and will produce a different band pattern to normal wild type. The PCR-SSCP method has been used for typing known SHV variants arising through specific point mutations in the \( \text{bla}_{\text{SHV}} \) gene. M’Zali et al.52 applied this method for detection of the gene encoding SHV-7 and for identification of different SHV genes within the same strain. The use of PCR-SSCP for characterization of isolates harboring \( \text{bla}_{\text{SHV-1}} \), \( \text{bla}_{\text{SHV-2a}} \), \( \text{bla}_{\text{SHV-4}} \), \( \text{bla}_{\text{SHV-5}} \), \( \text{bla}_{\text{SHV-11}} \) and \( \text{bla}_{\text{SHV-12}} \) was assessed by Chanawong et al.53. Edelstein and Stratchounski have digested the 1080bp \( \text{bla}_{\text{TEM}} \) amplicon with specific restriction endonucleases, which has allowed the use of PCR-SSCP technique for analysis of entire gene sequences and has permitted discrimination of 13 different genes encoding TEM-type \( \beta \)-lactamases.54

**Polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP).** The RFLP analysis enables characterization of DNA after digestion using restriction endonucleases and subsequent separation of DNA fragments in agarose gel. Restriction endonucleases are enzymes that cut double-stranded or single stranded DNA at specific recognition nucleotide sequences known as restriction sites. Discrimination of various DNAs is based on restriction fragment length polymorphism. This polymorphism occurs due to the presence or absence of restriction sites. The RFLP method is also simple and rapid for the detection of known mutations that alter recognition sites of restriction endonucleases. A large number of amino acid residues in particular are important for producing the ESBL phenotype in TEM \( \beta \)-lactamases when substitutions occur at specific positions. They include glutamate to lysine at position 104, arginine to either serine or histidine at position 164, glycine to serine at position 238, and glutamate to lysine at position 240.55 Arlet et al.56 used the PCR-RFLP method to detect single base mutations within \( \text{bla}_{\text{TEM}} \) gene that are responsible for amino acid substitutions affecting the phenotype. However, mutations involving amino acid substitution without extension of the spectrum of resistance have also been monitored. The simplest method for detection and identification of SHV derivates was proposed by Nüesch-Inderbinen.57 Due to the glycine (position 238) (SHV-non-ESBL) to serine (position 238) (SHV-ESBL) substitution, only PCR fragments from the genes coding for SHV-ESBLs were cleaved with \( \text{NheI} \) restriction enzyme. One way of distinguishing between the individual clusters of CTX-M enzymes is the digestion of CTX-M amplicons with \( \text{BseDI} \) endonuclease and subsequent gel electrophoresis. According to predicted patterns of DNA fragments, detected CTX-M enzymes can be grouped.58

**Ligase chain reaction (LCR).** LCR involves the use of two pairs of probes, each pair being complementary to a strand of the denatured target DNA. Each probe within a pair is designed to hybridize to adjacent stretches of DNA. If perfect hybridization occurs, particularly with the 3’ end of the upstream primer, then ligation between the two probes will proceed. If a mismatch occurs, ligation is inhibited as a result of imperfect hybridization of the probe with target DNA and thus the absence of a ligated product suggests the presence of at least a single-basepair mismatch within the probe sequence.59,60 Therefore, LCR is suitable for detecting point mutations, such as those occurring within the \( \text{bla} \) gene. Kim et al.61 used four sets of oligonucleotides for discriminatory detection of genes coding SHV variants such as SHV-1, 2, 2a, 3, 4, 5 and 12. Each primer detected aminoacid substitutions at positions 35 (glutamine for glutamic acid), 205 (leucine for arginine), 238 (serine for glycine) and 240 (lysine for glutamic acid), which allowed rapid characterization of clinical isolates sharing \( \text{bla}_{\text{SHV}} \) genes. Niederhauser et al.62, used ligase detection reaction – polymerase chain reaction (LDR-PCR) to test different ESBL-producing strains and clinical isolates for a specific point mutation in the \( \text{bla}_{\text{SHV}} \) gene (glycine to serine mutation at position 238) and compared this with the commercially available Etest ESBL. LDR-PCR proved to be a very sensitive technique since it was able to identify a single bacterium with a point mutation in a background of 100,000 wild-type (non-ESBL-producing) bacteria.

**Real-time PCR.** One genetic method used in practical medical diagnostics is the real-time PCR technique. This is due to the reduced cycle times, removal of separate post-PCR detection procedures and the use of sensitive fluorescence detection equipment, allowing earlier amplification detection. Unlike conventional PCR, real-time PCR allows continual monitoring of accumulating amplicon in real time by labeling primers, oligoprobes or amplicons with molecules capable of fluorescing. There are several types of detection (intercalating fluorescent dyes such as SybrGreen or LCGreen, dual-labeled probes, FRET probe systems) used in real-time amplification.

Real-time PCR with subsequent melting curve analysis has found a practical application in detection of SHV extended-spectrum \( \beta \)-lactamases. Randegger and Hächler showed it was a rapid, sensitive and specific method for detection of mutations in three crucial codons (amino acid positions 179, 238 and 240) of the \( \text{bla}_{\text{SHV}} \) gene in a single reaction. This method based on real-time PCR monitored with fluorescently labeled hybridization probes, followed by melting curve analysis was able to discriminate between \( \text{bla}_{\text{SHV-35/123-ESBL}} \) and \( \text{bla}_{\text{SHV-ESBL}} \) in clinical isolates. In a study by Chia et al.63, a combination of multiplex PCR plus real-time PCR and melting curve analysis were described for the specific and simultaneous identification of SHV and CTX-M type ESBLs. After optimization, the system was applied to characterize 199 clinical isolates of Enterobacteriaceae. The authors found that it could be easily adapted for similar studies in areas where different ESBLs are prevalent. For detection and genotyping of CTX-M-producing members of Enterobacteriaceae, Birkett et al.64 have developed a multiplex, real-time TaqMan PCR assay. In the view of the authors, the method could be useful locally for investigating epidemic outbreaks and would be suitable for use in regional or national reference facilities.
DNA microarray. The major disadvantage of the above methods is the limited number of targets that can be detected and differentiated in each reaction. DNA microarray seems to be a promising genotyping technique with a high multiplexing ability. Instead of detecting and studying one gene at a time, microarrays allow thousands or tens of thousands of specific DNA sequences to be detected simultaneously on a small glass or silica slide only 12 cm square. DNA microarrays are used in three major clinical areas: (a) for gene expression profiling – measuring the expression level of thousands of genes in any tissue sample, (b) for genotyping – determination of disease-relevant genes or agents causing diseases, and (c) DNA sequencing – screening thousands of DNA base pairs for mutations in specific genes whose normal sequence is already known (screening of single nucleotide polymorphisms, SNPs)\(^5\). Grimm et al.\(^5\) developed and validated oligonucleotide microarray for the rapid identification of ESBL in Gram-negative bacteria by simultaneously genotyping \(\text{bla}_{\text{TEM}}\), \(\text{bla}_{\text{SHV}}\), and \(\text{bla}_{\text{CTX-M}}\). The array consisted of 168 probes which cover mutations responsible for 156 amino acid substitutions with the assay time of 5 hours. A multiplex asymmetric PCR (MAPCR)-based microarray method was described by Zhu et al.\(^5\) for detection of 10 known ESBLs in Enterobacteriaceae and for typing of six important point mutations (amino acid positions 35, 43, 130,179, 238 and 240) in the \(\text{bla}_{\text{SHV}}\) gene.

Direct sequencing. Direct sequencing remains the gold standard for identifying the unknown products of amplification reactions. Instruments are now available for semi-automated running and analyzing of sequence gels. Thus, any resistance gene or resistance mutation can be determined using this method\(^5\).

Molecular genetic methods used for monitoring of clonal dissemination of ESBL-producing isolates and horizontal transfer of the determinants of resistance

In 1983, the ESBL phenomenon was first reported in Germany\(^5\). Soon after, extended-spectrum enzymes increased in number and variety and expanded worldwide. Their prevalence varies from one country to another and from hospital to hospital. The epidemiological characteristics of ESBL outbreaks indicate that the high prevalence can be explained by clonal dissemination of ESBL-producing strains as well as horizontal transfer of \(\text{bla}\) genes on various mobile genetic elements among non-related isolates.

These mobile genetic elements can be divided into two general types: (I) elements that can move from one bacterial cell to another (resistance plasmids and conjugal resistance transposons), and (II) elements that can move from one genetic location to another in the same cell (resistance transposons, gene cassettes and ISCR-promoted gene mobilization)\(^5\). Every year, numerous studies from different countries are published describing genetic detection of ESBL-producing strains focused on characterization of transferable elements involved in horizontal transfer between sensitive and resistance populations. The most used techniques include preparation of plasmids either by a modified alkaline-lysis method\(^5\) or using commercial isolation kits based on various modification of this method, classification into plasmid incompatibility groups, followed by characterization of present plasmid DNA using restriction analysis, Southern blotting, hybridization with specific probes, partial plasmid sequencing or transfer of these mobile elements by conjugation and transformation experiments\(^6\). For characterization of integrons and their classification into classes or present gene cassettes, various PCR primers have been described\(^61,63\). The PCR technique is also used for exact description of genetic environment of \(\text{bla}\) genes and determination of different insertion sequences involved in mobilization and expression of relevant genes\(^64\).

Many investigators use genetic methods to examine epidemiology of the strains involved in the outbreaks. Although not all molecular techniques are equally effective for typing all organisms, pulsed-field gel electrophoresis (PFGE) based on restriction analysis of genomic DNA currently seems to be the best available method for most pathogens. Other techniques that differ in reproducibility, discriminatory power, simplicity of interpretation or performance include analysis of plasmid DNA, ribotyping, insertion sequence typing, PCR-based methods such random amplified polymorphic DNA PCR (RAPD-PCR), PCR of repetitive chromosomal elements (Rep-PCR), PCR restriction fragment length polymorphism (PCR-RFLP), infrequent restriction site PCR (IRSPCR), amplified fragment length polymorphism (AFLP), enterobacterial repetitive intergenic consensus (ERIC) fingerprinting, multilocus sequence typing (MLST) and others\(^69-71\).

PRACTICAL EXPERIENCES WITH MOLECULAR GENETIC METHODS IN ESBL DETECTION

Our first study to use modern genetic methods for ESBL detection focused on the molecular genetic analysis of ESBL-positive isolates of Klebsiella pneumoniae in intensive care patients\(^72\). For characterization of acquired strains, the PCR technique followed by RFLP analysis was used. Restriction cleavage with \(\text{NheI}\) revealed mutations at position 238 in all SHV-positive isolates. The PFGE method that we used for molecular typing of resistant strains detected low level of clonal spread at the Department of Anesthesiology and Resuscitation of the University Hospital Olomouc, suggesting both rational antibiotic policy and high levels of hygienic and epidemiological measures at the department. In another study, we focused on determining the prevalence of P. aeruginosa and K. pneumoniae strains in patients with acute leukemias, and on assessing the clinical significance of this\(^73\). Using PFGE we tried to define the sources and means of spreading. We found that analysis of individual clones can help to characterize the prevalence of these clones and thus characterize probable representation of endogenous and exogenous infections. For molecular-genetic characterization of ESBL-positive Enterobacteriaceae from the gastrointestinal tract of non-hospitalised peo-
ple living in the Olomouc Region of the Czech Republic. PCR followed by RFLP analysis was used\textsuperscript{14}. The results were confirmed using direct sequencing and CTX-M-15 β-lactamase was defined as the most prevalent ESBL type in our community. Some ESBL-positive strains have also been recovered from domestic farm animals in the Czech Republic\textsuperscript{75}. In a broiler group (154 composite samples), one ESBL-positive \textit{Escherichia coli} producing CTX-M-14 β-lactamase was identified. Of a total of 150 turkey cloacal swabs, six ESBL-positive \textit{Escherichia coli} strains with the production of SHV-12 and CTX-M-1 enzymes were obtained. PFGE detected one group of three and one group of two identical strains of ESBL-positive \textit{E. coli} isolated from turkeys in a single farm, suggesting their clonal spread. In our last study, we tested real-time PCR method with melting curve analysis as a rapid tool for identification and discrimination of present extended-spectrum enzymes, especially those of the CTX-M family. Our data show that the prevalence of ESBL-positive \textit{E. coli} in the gastrointestinal tract was 1.2\% (ref.\textsuperscript{74}) in non-hospitalised people living in the community and 1.1\% in hospitalized patients, with similar representation of CTX-M types in both groups. The hospital and community isolates were found to be genetically unrelated. The results suggest that this method may be suitable for epidemiological studies in the area of bacterial resistance.

ADVANTAGES AND LIMITATIONS OF GENETIC METHODS

Prior to conventional phenotypic methods to determine antimicrobial susceptibility, the pathogen has to be isolated from human specimens by culture methods taking at least 24 hours and then exposed to various concentrations of antimicrobial agents using diffusion or dilution tests to determine the ability of these agents to inhibit its growth. Genetic methods assessing the genotype of the microorganism, not its phenotype (expression of this genotype), can often be performed directly with clinical specimens, obviating the need for isolation. Since some organisms cannot be cultured or are difficult to culture, determination of the relevant phenotype may take long or it may not be determined at all. Conventional methods may also fail due to the resistance mechanisms involved in low-level resistance because of detection problems with poor expression. One of the advantages of genetic methods is that they allow reduction of the biohazard risks associated with conventional culture methods. These methods are therefore characterized by higher detection ability and are capable of reducing the detection time and subsequent susceptibility interpretations. Unlike for example pulsed-field gel electrophoresis and plasmid analysis, no method based on phenotype determination can analyze the spread of resistant microorganisms or resistance determinants throughout the population.

On the other hand, therapeutic decisions are preferably based on detection of susceptibility since microorganisms showing positive results in genotypic tests may remain phenotypically susceptible to certain antimicrobial agents due unexpressed resistance genes. Moreover, these genes may be expressed at such low levels that their effects are clinically irrelevant. Also, since genetic methods do not take into account new resistance mechanisms, culture methods are useful for detecting emerging or new forms of antimicrobial resistance. False-positive results may occur because of contamination of the test sample with extraneous nucleic acid or residual nucleic acid from prior samples or due to presence of silent genes and pseudogenes.

Although genetic techniques seem to be suitable for detection of antimicrobial resistance they are unlikely to replace the traditional cheaper and flexible phenotypic tests in the nearest future. However, they can be helpful in providing explanations for unusual, complex antibiograms, confirming specific resistance mechanisms and in rapid identification and detection of resistance mechanisms in slow-growing organisms such as \textit{Mycobacterium tuberculosis}. These modern methods have great potential for epidemiological studies that monitor the spread of resistant strains and mobile genetic elements in human or animal populations\textsuperscript{6–8}.

CONCLUSIONS

In recent decades, a large number of molecular techniques have been developed for detection of serious microbial pathogens and their resistance or pathogenic markers. While many have found practical application in routine microbiological diagnostics, others are currently used in research only. Currently, one of the main disadvantages are high costs, including those of equipping of molecular genetic laboratories, especially in local hospitals and their diagnostic centers. On the other hand, if these modern methods are introduced into diagnostics, they often help in rapid and accurate detection of certain microorganisms or their resistance and pathogenic determinants. Genetic methods have a great potential in examination of strains involved in disease outbreaks and in identification of the epidemic clones. At our department, several genetic methods are used for detection and further characterization of extended-spectrum β-lactamases. However, these are mostly research techniques used for confirmation of phenotypic detection, currently the gold standard in microbiological determination of these enzymes. Therefore, it will take time for the molecular genetic methods to become an integral part of all areas of medical microbiology detection.

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Extended Spectrum ß-Lactamase (ESBL) Expression in Escherichia coli Isolates from Anal Swabs of Donkeys in a Local Donkey Abattoir in Abakaliki, Nigeria. 04. Results. 

Authors The extended spectrum ß-lactam (ESBL) producing strains of Enterobacteriaceae with diverse types of ESBL genes are resistant to antibiotics, which create serious global health problem especially in the hospital, set up. The mechanisms of ESBL production among Escherichia coli, Klebsiella species and Enterobacteriaceae are discussed. A negative bacteria which produce extended spectrum beta-lactamases (ESBLs). The Ent erob act eriaceae is the largest family of Gram-negative, rod shaped, non fermenting facultative, anaerobic bacteria.
The PCR method with detection of the results in the real time regimen (PCR test-system Litekh) provided detection of the beta-lactamase genes: bla/CTX-M-like (72/104, 69.2%), blaNDM (6/104, 5.8%), blaVIM (49/104, 47.1%) and blaOXA48-like (59/104, 56.7%). There was identified correlation between the phenotype of resistance of Acinetobacter spp., Pseudomonas aeruginosa, Klebsiella pneumoniae and Escherichia coli to cefotaxime and carbopenems and detection of the blaCTX_M-like and blaNDM genes.  

3. Lynch J.P., 3rd, Clark N.M., Zhanel G.G. Evolution of antimicrobial resistance among Enterobacteriaceae (focus on extended spectrum beta-lactamases and carbapenemases). Expert Opin Pharmacother 2013; 14: 2: 199-210. Horizontal gene transfer is the most common mechanism associated with the spread of extended-spectrum ß-lactam- and carbapenem resistance among pathogenic bacterial species. Along with the increase in antimicrobial resistance, many different types of ESBLs and carbapenemases have emerged with different enzymatic characteristics. For example, carbapenemases are represented across classes A to D of the Ambler classification system.  

To date, ß-lactamases have been classified based on the molecular structure classification of Ambler [16] and the functional classification of Bush-Jacobi-Medeiros (Fig. 5) [17, 18]. Extended-spectrum ß-lactamases (ESBL) are enzymes that confer resistance to most ß-lactam antibiotics, including penicillins, cephalosporins, and the monobactam aztreonam. Infections with ESBL-producing organisms have been associated with poor outcomes. Community and hospital-acquired ESBL-producing Enterobacteriaceae are prevalent worldwide [1]. Reliable identification of ESBL-producing organisms in clinical laboratories can be challenging, so their prevalence is likely underestimated. Carbapenems are the best antimicrobial agent for infections caused by such organisms. The types and de...