INTRODUCTION

Multidrug resistance is now emerging worldwide at an alarming rate among Gram negative bacteria, causing both community-acquired and nosocomial infections ***(Schwaber et al., 2006).*** One of the most important emerging resistance traits in *Enterobacteriaceae* family members corresponds to resistance to broad-spectrum β-lactam antibiotics, which is mainly associated with production of extended-spectrum β- lactamase (ESBL) enzymes ***(Pitout and Laupland, 2008 and Coque et al., 2011).***

ESBLs are typically plasmid-mediated enzymes that hydrolyze the penicllins, the third generation cephalosporins (cefotaxime, ceftriaxone, ceftazidime) and the monobactam aztreonam. They are not active against the cephamycins (cephoxitin and cefotan) and carbapenems (imipenem, meropenem) but are susceptible to β- lactamase inhibitors such as clavulanic acid and tazobactam ***(Pfaller and Segreti, 2006).***

In the context of world wide spread of multidrug resistance, ESBL producers that are mostly *E.coli* and *Klebsiella pneumoniae* species are not only found as source of nosocomial but also of community acquired infections ***(Coque et al., 2008; Pitout and Laupland 2008 and Poirel et al., 2012).***

A variety of ESBLs, mostly of the genotypes CTX-M, TEM and SHV types, had been reported in members of *Enterobacteriaceae* family ***(Poirel et al., 2012).***

The ESBLs often remain undetectable by the current isolation and susceptibility methods ***(Norman et al., 20l2).***

Current techniques for detecting ESBL producers are based on the determination of susceptibility to broad–spectrum cephalosporins followed by inhibition of the ESBL activity, mostly by clavulanic acid or tazobactam.

The double- disk synergy test and the E-test were proposed for that purpose with 80% to 90 % sensitivities and specificities ***(Drieux et al., 2008 and Gazin et al., 2012).*** Based on the same principle, automated methods for bacterial identification and susceptibility testing are also used for the detection of ESBL producing bacteria with a sensitivity range 80–90% and 50-80% specificity ***(Drieux et al., 2008).***

The previous methods require overnight growth, meaning that up to 24 to 48 hrs can elapse before ESBL production is detected once the isolate had grown ***(Drieux et al., 2008 and Gazin et al., 2012).*** This may conduce to a delay in the initiation of appropriate antibiotic therapy ***(Schwaber et al., 2006).***

Molecular detection of ESBL genes by PCR, hybridization, and sequencing is an alternative but remains costly and requires a certain degree of expertise that is not accessible to non-specialized laboratories.

As PCR-based techniques require isolation of the organism from clinical specimens, the results cannot be obtained until 48hrs after obtaining the pathological samples ***(Gazin et al., 2012).***

Recently, a rapid and cheap biochemical test was developed for the detection of ESBL producers, namely ESBL NDP test. This test is based on a technique designed to identify the hydrolysis of the β-lactam ring of a cephalosporin (cefotaxime), which generates a carboxyl group, by acidifying a culture medium. The acidity resulting from this hydrolysis is identified by the color change generated using a ph indicator such as phenol red ***(Norman et al., 2012).***

AIM OF THE WORK

The aim of this study was to evaluate the performance of ESBL NDP test for rapid detection of ESBL-producing *E.coli* in order to choose an appropriate antimicrobial therapy for patients infected with such bacteria.

ANTIBIOTICS

In 1928, Alexander Fleming discovered penicillin, the first chemical compound with antibiotic properties. He was working on a culture of bacteria when he noticed the spores of little green mold in one of his culture plates. He observed that the presence of the mold prevented the growth of the bacteria. Antibiotics have greatly revolutionized medicine in the 20th century. Together with vaccination, they have nearly caused the eradication of diseases such as tuberculosis in the developed world. Unfortunately, their effectiveness and easy access led to overuse, causing bacteria to rapidly develop resistance to a wide range of antibiotics. In response, the World Health Organization classified antimicrobial resistance as a serious threat with serious outcomes on the health of the population worldwide ***(WHO, 2014).***

Antibiotics are mainly used for the treatment or prevention of bacterial infections. They are administered for the treatment of infection as part of an empirical therapy or definitive therapy**.** Antibiotics may also be given prophylactically to prevent infection. Use of prophylactic therapy should be limited to populations at high risk of developing infection due to weakened immune systems, to minimize the emergence of resistance as possible ***(Jones and Bartlett, 2011).***

At risk populations may include patients on immuno-suppressive therapy, patients with cancer or who are having major surgery ***(Jones and Bartlett, 2011).***

Antibiotics are often classified based on their chemical structure or their mechanism of action. They are either bactericidal or bacteriostatic ***(Pelczar et al., 1999).***

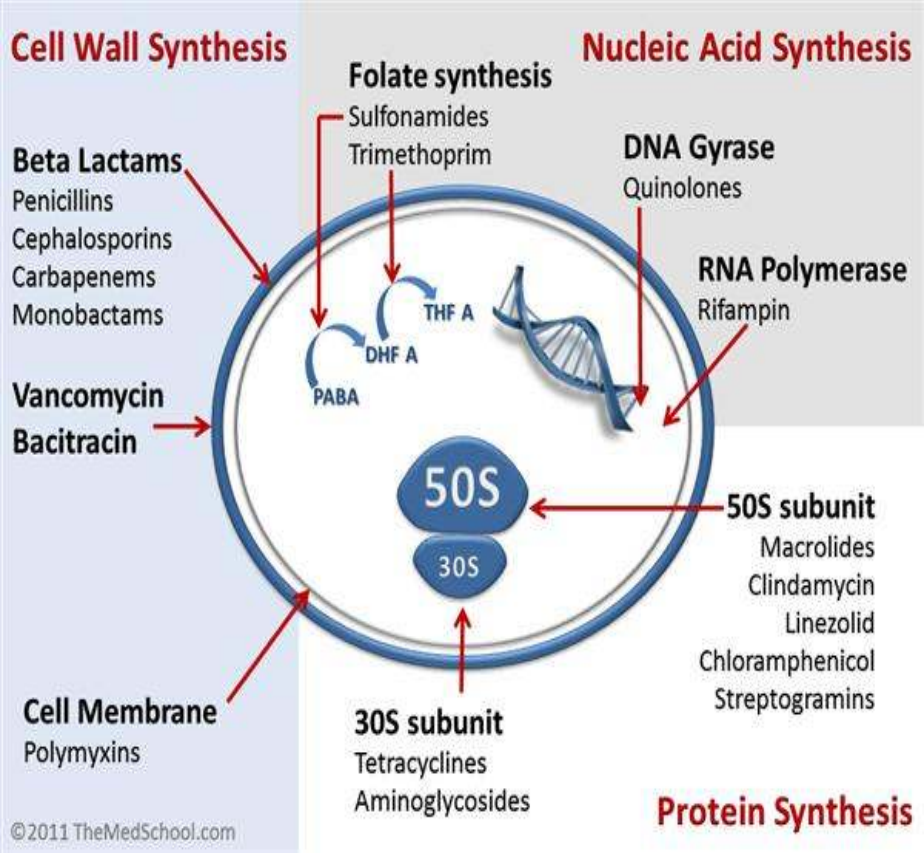

Figure (1): Mechanisms of action of different antibiotics
(The Medschool, 2011).

Classification of antibiotics according to the mechanism of action *(Berger, 2014):*

1. Cell Wall Synthesis inhibitors:

These drugs are usually bactericidal. They include drugs that block cross linking by competitive inhibition of the transpeptidase enzyme as penicillins, cephalosporins, β-lactamase inhibitors, carbapenems & aztreonam. Vancomycin also inhibits cell wall synthesis by disruption of the cross linking. Bacitracin interferes with the dephosphorylation of C_{55}-isoprenyl pyrophosphate, a molecule that carries the building-blocks of the peptidoglycan bacterial cell wall outside of the inner membrane. This group also includes the mycolic acid synthesis inhibitor, isoniazid, which is an anti-tuberculous drug.

A- β-lactam antibiotics:

β-lactam antibiotic family comprises all agents that contain a β-lactam ring in their molecular structure, including derivatives of penicillin, cephalosporins, monobactams, and carbapenems ***(Holten and Onusko, 2000).*** β-lactam antibiotics mostly work by inhibiting cell wall biosynthesis in the bacterial organism. They are different from each other by the chemical side chain attached to their nuclei. The β-lactam ring is crucial for antibacterial activity while the side chain determines the antibacterial spectrum and pharmacologic properties ***(Mandell et al., 2000).***

β-lactams are the most widely used antibiotics due to their relatively high effectiveness, low cost, easy delivery and few side effects. β-lactam antibiotics are bactericidal as they act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls ***(Livermore, 2008).***

The peptidoglycan layer is crucial for the integrity of the structure of the cell wall, mainly in Gram-positive bacteria, as it's the outermost and main component of the cell wall. The final transpeptidation step in the synthesis of the peptidoglycan is mediated by DD-transpeptidases, known as penicillin-binding proteins (PBPs). PBPs differ in their affinity for binding penicillin & other β-lactam antibiotics & their amounts vary among different bacterial species.

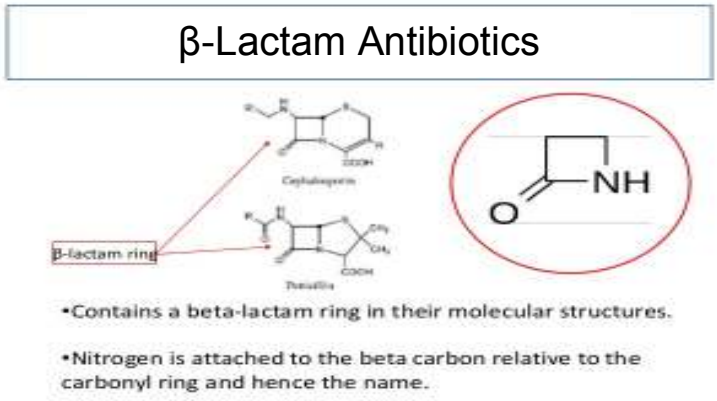

Figure (2): Structure of β-lactam antibiotics ***(Brian, 2001).***

β-lactam antibiotics are structurally similar to d-alanyl-d-alanine, the terminal amino acid residues on the precursor N-acetyl muramic acid (NAM) & N-acetyl glutamic acid (NAG)

peptide subunits of the newly synthesized peptidoglycan layer. This facilitates their irreversible binding to the Ser_{403} residue of the active site of PBPs, inhibiting the cross-linkage & leading to disruption of the cell wall synthesis.

Normally the precursors of peptidoglycan give the signal to reorganize the bacterial cell wall, leading to triggering the activation of autolytic cell wall hydrolases. Inhibition of cross-linkage by β-lactams leads to accumulation of the peptidoglycan precursors, which in turn stimulates the digestion of existing peptidoglycan by autolytic hydrolases without the production of new peptidoglycan. This further enhances the bactericidal action of β-lactam antibiotics ***(Fisher et al., 2005).***

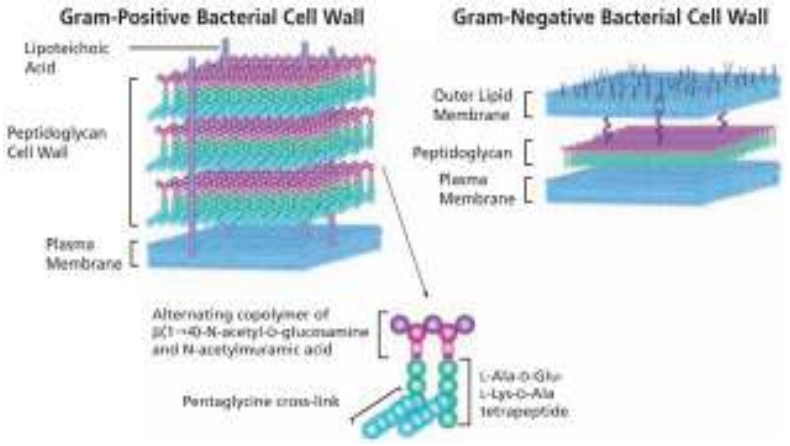

Figure (3): Cell wall structure of Gram-positive & Gram-negative bacteria (Sigma-Aldrich)

Penicillin binding proteins are localized to the outer leaflet of the bacterial cytoplasmic membrane, rendering them relatively accessible to the action of β-lactams.

Furthermore, penicillin binding proteins are specific to the bacteria accounting for the low toxic effects of this class of antibiotics ***(Livermore, 2008).*** The commonly used β-lactam antibiotics are the penicillins, the cephalosporins, the carbapenemes & the monobactams.

I. Penicillins (Penams):

British scientist Alexander Fleming discovered penicillin, the first antibiotic in the world, in 1928 as a natural product from Penicillium mold. Penicillins remain a primary choice for treatment of different bacterial infections in spite of the discovery of many new classes of antibiotics ***(Arnold and Sanchez, 2009).***

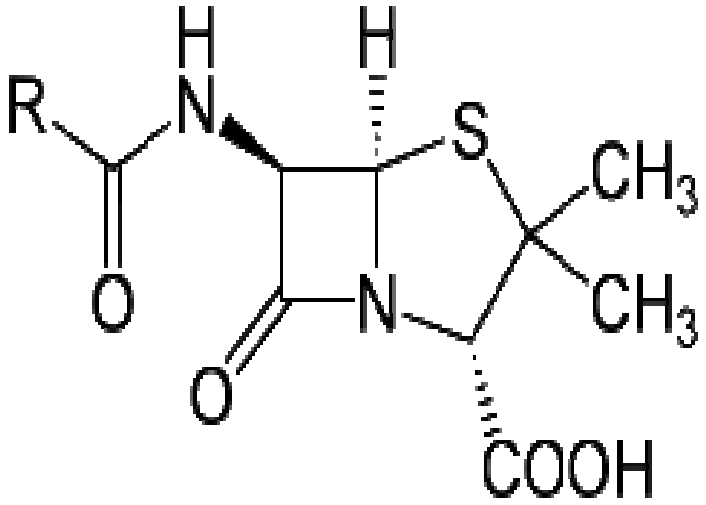

Figure (4): Penicillin core structure, where "R" is the variable group ***(Brian, 2001).***

Classification of Penicillins:

a) Natural Penicillins:

Natural penicillins are the first clinically used agents in the penicillin family. They are directly acquired from the *Penicillium* mold with no further modification. They include penicillin G, procaine penicillin, penicillin V, and benzathine penicillin.

They are effective against gram-positive cocci and some gram-negative cocci such as meningococci. The natural penicillins are highly susceptible to inactivation by β-lactamases ***(Drews and Jürgen, 2000)***.

b) Penicillinase-Resistant Penicillins:

Methicillin was the first drug of this class. After that, oxacillin, nafcillin, cloxacillin and dicloxacillin were added.

Though these penicillins don't have a broad spectrum activity compared to the natural penicillins, they are highly effective against penicillinase-producing strains of Gram positive cocci, particularly staphylococci. That's why these penicillins are often called as anti-staphylococcal penicillins ***(Cunha, 2009*)**.

c) Aminopenicillins:

These penicillins were the first to possess activity against Gram negative bacteria. They are acid resistant so they can be introduced orally. This family includes amoxicillin, bacampicillin

and ampicillin. Unfortunately, these drugs are vulnerable to β-lactamases ***(Cunha, 2009).***

d) Extended Spectrum Penicillins:

They are also called anti-pseudomonal penicillins for their effectiveness against *Pseudomonas aeruginosa* in addition to their activity against many other Gram negative bacteria. They include both acylaminopenicillins (mezlocillin, piperacillin, and azlocillin) and alpha-carboxypenicillins (ticarcillin and carbenicillin). They are commonly co-administered with aminoglycosides. These drugs are also vulnerable to inactivation by β-lactamases, similar to the aminopenicillins ***(Leon, 2009)***.

II. Cephalosporins (Cephems):

Cephalospoins are derived from *Acremonium* (*Cephalosporium*) fungus & were discovered in 1945. Nowadays, they are considered the most widely used antibiotics. They constitute together with cephamycins a subgroup of β-lactam antibiotics called cephems.

Cephalosporins are also commonly used for surgical prophylaxis and prevention of bacterial infection before, during, and after surgery ***(Pichichero, 2006).***

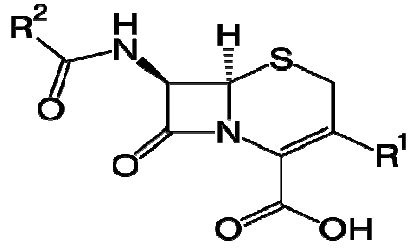

Figure (5): Cephalosporin core structure ***(Brian, 2001).***

Classes of Cephalosporins *(Tumah, 2005 and Kollef, 2009):*

a) First Generation Cephalosporins

They have a relatively narrow spectrum of activity, mainly on Gram-positive bacteria with poor efficacy against the Gram-negatives. They include cephalothin, cefazolin, cephapirin, cepadrine, cephalexin, and cefadroxil. This class is vulnerable to inactivation by β-lactamases.

b) Second Generation Cephalosporins

Their gram-negative spectrum is greater than drugs of the first generation in addition to retaining some activity against Gram-positive bacteria. Moreover, they are more resistant to β-lactamases. This class comprises two groups with similar activity:

1. The true 2nd generation cephalosporins (cefaclor, cefamandole, cefprozil and cefuroxime)
2. The cephamycins (cefmetazole, cefotetan and cefoxitin). Cephamycins are generally resistant to β-lactamases owing to their 7-alpha-methoxy group that differentiates them from other cephalosporins.

Moreover, cephamycins differs from the 2nd generation drugs in their strong effectiveness against anaerobes.

c) Third Generation Cephalosporins

These drugs have greater effectiveness against Gram-negative bacteria than agents of the 2nd generation. They can cross the blood brain barrier. They are inactivated by β-lactamases.

Third generation drugs include cefotaxime, moxalactam, cefoperazon, ceftiaoxime, ceftazidime, ceftriaxone, cefitofur, and cefixime.

d) Fourth Generation Cephalosporins

They have the widest spectrum of activity. They are effective against Gram-negative bacteria while retaining activity against the Gram-positives similar to the first generation. They are more stable against β-lactamases than the third generation cephalosporins. This class comprises cefepime and cefpirome. They are usually reserved for treating resistant infections.

e) Fifth Generation Cephalosporins

This class includes one agent, ceftobiprole. Ceftobiprole is a very broad-spectrum cephalosporin with activity against gram-positive cocci, including methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus epidermidis* (MRSE), penicillin-resistant *Streptococcus pneumoniae*, *Enterococcus faecalis* and many gram-negative bacilli including AmpC producing *E. coli* and *Pseudomonas aeruginosa*.

f) Advanced- generation cephalosporin:

Ceftaroline is a new advanced-generation cephalosporin agent which is similar to ceftobiprole in its effectiveness against multidrug-resistant *Staphylococcus aureus*, including MRSA, VRSA, and VISA, and *Enterococcus*.

III. Carbapenems and Penems:

Carbapenems are considered the last resort antibiotics for severe bacterial infections, usually in critically ill patients, especially those caused by the *Enterobacteriacae* ***(Stephen, 2010).*** They have a broad spectrum of activity, being effective against both Gram-positive & Gram-negative bacteria and anaerobes. They are unique in their ability to inhibit L-D-transpeptidases. However they are ineffective against intracellular bacteria as *Chlamydia*. They are stable against AmpC β-lactamases & ESBLs ***(Mainardi et al., 2008).***

Resistance to carbapenems is usually mediated by production of the New Delhi Metallo-β-lactamase 1(NDM-1). Antibiotic options are limited for treatment of carbapenem-resistant bacteria ***(Temkin et al., 2014).*** Therefore carbapenems should be reserved for resistant infections caused by bacteria proved to be non-susceptible to other antibacterial agents ***(Rello et al., 2004).***