A Review on Penicillinase Production and Beta-Lactamase Activity on Commonly Isolated Bacteria

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ABSTRACT

Beta-lactamases are enzymes produced by some bacteria allow in them to provide resistance to beta-lactam antibiotics like penicillins, cephamycins, and carbapenems (Ertapenem), although carbapenems are relatively resistant to beta-lactamase. Extended Spectrum Beta-lactamase (ESBL) has the ability to hydrolyze and cause resistance to various types of the newer β-lactam antibiotics. Penicillinase is a specific type of β-lactamase, showing specificity for penicillins, again by hydrolysing the beta-lactam ring. Penicillinase-resistant beta-lactams such as methicillin were developed, but there is now widespread resistance to even these antibiotics. Currently, ESBLs are becoming a major threat for patients in the hospital, long-term care facilities, and community. These bacteria have not only caused outbreaks but have become endemic in many hospitals throughout the world. It is therefore necessary to place surveillance of antibiotics in our Society to avoid increase antibiotics resistance producing organisms. Routine monitoring of antibiotic resistance to provides data for antibiotic therapy and resistance control with information will directly affect selection of empiric therapy for urinary tract infection in pregnant women is recommended to promote good antenatal management in the health sector. The awareness of the existence of ESBL is recommended to initializes indication for the need for proper use of antibiotics and spread of multi drug resistance bacterial strains within these hospital and communities.

Keywords: Penicillinase, Beta-Lactamase, Bacteria, ESBL, beta-lactam, Methicillin.

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INTRODUCTION

Beta-lactamases are enzymes produced by some bacteria allow in them to provide resistance to beta-lactam antibiotics like penicillins, cephamycins, and carbapenems (ertapenem), although carbapenems are relatively resistant to beta-lactamase. Beta-lactamase provides antibiotic resistance by breaking the antibiotics' structure [1,2]. These

antibiotics all have a common element in their molecular structure: a four-atom ring known as a beta-lactam. Through hydrolysis, the lactamase enzyme breaks the β-lactam ring open, deactivating the molecule's antibacterial properties. Beta-lactam antibiotics are typically used to treat a broad spectrum of Gram-positive and Gram-negative bacteria. Beta-lactamases produced by Gram-negative organisms are usually secreted, especially when antibiotics are present in the environment used for conventional treatment of bacterial infection [3].

Resistance to antibiotics has become a global burden. Microorganism confers resistance via several means. Organisms producing beta lactamase enzymes remain an important reason for therapy failure with cephalosporin class of antibiotics and have serious consequences for infection control [4,5]. Resistance to older generations of antimicrobials is high in most areas and resistance to most new antimicrobials has appeared in community acquired uropathogens [6]. Bacteria such as *Escherichia coli*, a Gram-negative, facultative anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms) which conferred β-lactamases enzymes activities that inactivate β-lactam antibiotics by hydrolysis, which results in ineffective compounds [1,7]. One group of β-lactamases, i.e. extended-spectrum β lactamases (ESBLs), have the ability to hydrolyse and cause resistance to various types of the newer β-lactam antibiotics, including the extended-spectrum (or third-generation) cephalosporins (cefotaxime, ceftriaxone and ceftazidime) and monobactams (aztreonam), but not the cephamycins (cefoxitin and cefotetan) and carbapenems (imipenem, meropenem and ertapenem) [1]. Currently, ESBLs are becoming a major threat for patients in the hospital, long-term care facilities, and community. These bacteria have not only caused outbreaks but have become endemic in many hospitals throughout the world. When a significant proportion of Gram-negative isolates in a particular unit are ESBL producers, empirical therapy may change towards use of imipenem, quinolones, or b-lactam/β-lactamase inhibitor combinations [6]. In some centers this has been associated with emergence of imipenem resistance in *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, and in ESBL-producing organisms themselves [1,6].

The control of endemic ESBL producers is difficult, and

may only be possible after significant nursing and medical reorganization with research, at substantial financial cost [8]. Patients at high risk for developing colonization or infection with ESBL producing organisms are often seriously ill patients with prolonged hospital stays and in whom invasive medical devices are present (urinary catheters, endotracheal tubes, central venous lines) for a prolonged duration [8]. A myriad of other risk factors have been implicated including the presence of nasogastric tubes, gastrostomy or jejunostomy tubes and arterial lines, administration of total parenteral nutrition, recent surgery, haemodialysis, decubitus ulcers, and poor nutritional status. Heavy antibiotic use is also a risk factor for acquisition of an ESBL-producing organism [3]. A strong relationship exists between third-generation cephalosporin use and acquisition of an ESBL producing strain [3]. Other antibiotic classes that have been found to be associated with subsequent infections due to ESBL-producing organisms include quinolones, trimethoprim- sulphamethoxazole, aminoglycosides and metronidazole [2,8].

Escherichia coli conferred beta-lactam antibiotics resistance used for treatment of bacterial infection and relates the term extended spectrum beta lactamase (ESBLs) producing bacteria which are beta-lactamases capable of conferring bacterial resistance to the penicillins, first-, second-, and third-generation cephalosporins and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics, and are inhibited by B-lactamase inhibitors such as clavulanic acid (Paterson et al., 2013). This was first described in Germany in 1983 and France in 1985 among Klebsiella spp [2]. ESBLs exist in every region of the world and in most genera of enterobacteria [6,8].

Beta-lactamases are enzymes produced by some bacteria (*Escherichia coli*) allowing them to provide resistance to beta-lactam antibiotics like penicillins, cephamycins, and carbapenems (ertapenem), although carbapenems are relatively resistant to beta-lactamase. Beta-lactamase provides antibiotic resistance by breaking the antibiotics' structure [1]. These antibiotics all have a common element in their molecular structure: a four-atom ring known as a beta-lactam. Through hydrolysis, the lactamase enzyme breaks the β-lactam ring open, deactivating the molecule's antibacterial properties. Beta-lactam antibiotics are typically used to treat a broad spectrum of Gram-positive

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Beta-Lactamase

Beta-latamase are enzymes produced by some bacteria that provide resistance to beta-lactam antibiotics like penicillins, cephamycins, and carbapenems (ertapenem) [3], although carbapenems are relatively resistant to beta-lactamase. Beta-lactamase provides antibiotic resistance by breaking the antibiotics' structure [9]. These antibiotics all have a common element in their molecular structure: a four-atom ring known as a beta-lactam. Through hydrolysis, the lactamase enzyme breaks the β-lactam ring open, deactivating the molecule's antibacterial properties [10]. Beta-lactam antibiotics are typically used to treat a broad spectrum of Gram-positive and Gram-negative bacteria [3,10].

The first antibiotic was discovered by Sir Alexander Fleming in 1927 and was named penicillin, which is a beta-lactam. It was not until the early 1940s, through the work of Florey, Chain and Heatley, that penicillin was purified and shown to cure specific bacterial infections [3]. Since that time, many chemical derivatives have been developed from penicillin to combat resistance that has arisen in bacteria. These derivatives commonly referred to as the extended-spectrum beta-lactams, (ESBL) include antibiotics called cephalosporins, carbapenems and monobactams [3,11].

Emergence of Extended Spectrum β-Lactamase (ESBL)

Extended Spectrum β-lactamase (ESBL) producing organisms first reported in Germany in 1983 and outbreaks of infections due to these organisms soon occurred in several European and African countries [2]. Organisms producing ESBL are clinically relevant and remain an important cause for failure of therapy such as Cephalosporin in treatment of ailment caused by bacteria [12]. Indeed the failure of cephalosporin therapy in an infection where the pathogen was reported to be susceptible to the drug in routine susceptibility testing has often been first indicator that the infecting strains produce an ESBL [2]. ESBL are often encoded by gene located on large plasmid and these also carry gene for resistance to other antimicrobial agents such as Aminoglycosides, Sulphonamide, Tetracycline and Chloramphenicol [1]. The emergence of resistance to B-lactam antibiotics began even

before the first B-lactam, Penicillin was developed. The first beta-lactamase was identified in *Escherichia coli* prior to the release of penicillin for use in the medical practice [13]. The age of penicillin saw the rapid emergence of resistance in *Staphylococcus aureus* due to the plasmid encoded penicillin [8].

Over the last 20 years, many new beta-lactamase antibiotics have been developed that were specifically designed to be resistant to the hydrolytic action of β-lactamase. However, with each new class that has been used to treat patients, new B-lactamase emerged that caused resistance to that class of drug. Presumably, the selective used and overused of the new antibiotic in the treatment has selected for new variants of β-lactamases [13]. One of these new classes was the cephalosporin otherwise known as the extended spectrum B-lactamase antibiotics which became widely used for the treatment of serious infection due to Gram negative bacterial in the 1980s. Not surprisingly, resistance to those extended spectrum β-lactamase antibiotics due to β-lactamase emerged quickly. Today over 150 different ESBLs have been described [2]. These β-lactamase have been found worldwide in many different general of *Enterobacteriaece* and *Pseudomonas aureginosa* [3]. The treatment of *E. coli* infection is increasingly becoming difficult because of the multi-drug resistance exhibited by the organism. Extended spectrum B-lactamase (ESBL) producing organism pose a major problem for clinical therapeutics.

Mechanism of Beta-Lactamase

Beta-lactam antibiotics kill bacteria by stopping the synthesis of the bacterial cell wall. As the contents inside a bacterial cell are much different from the outside environment, a wall needs to be produced which provides structure that prevents the cell from bursting. The beta-lactam antibiotics bind to the components that build this wall and inactivate them. Thus, bacteria can no longer produce the cell wall and they burst and die. To counteract the effects of the beta-lactam antibiotics the bacteria have evolved enzymes called the beta-lactamases which break down the beta-lactam drugs. Thus, they are capable producing the cell wall even in the presence of the beta-lactam drugs and are classified as resistant [1].

As the bacteria developed resistance to one type of beta-lactam antibiotic, new antibiotic derivatives were made by researchers and were called the cephalosporins, carbapenems

and monobactams, but the bacteria continually evolved and changed existing beta-lactamase enzymes to break down these new compounds [11]. The enzymes that can break down the newer derivatives are known as the extended-spectrum beta-lactamases (ESBLs) [8]. ESBLs can be found in many different types of bacteria and thus infections caused by ESBLs producing bacteria are numerous. Infections can involve the respiratory tract (pneumonia), urinary tract and bladder, skin and soft tissue, blood, gastrointestinal tract, reproductive organs, and central nervous system. Generally, individuals with infections caused by bacteria carrying ESBLs have a higher mortality rate and require longer stays in hospitals [8].

Some Beta-Lactamase Bacteria

Escherichia coli

Escherichia coli belong to bacterial family, *Enterobacteriaceae*, the enteric bacteria, which are facultative anaerobic Gram-negative rods that live in the intestinal tracts of animals in health and disease. The Enterobacteriaceae are among the most important bacteria medically. A number of genera within the family are human intestinal pathogens (e.g. *Salmonella, Shigella, Yersinia*). Several others are normal colonists of the human gastrointestinal tract (e.g. *Escherichia, Enterobacter, Klebsiella*), but these bacteria, as well, may occasionally be associated with diseases of humans [14,15].

Physiologically, *E. coli* is versatile and well-adapted to its characteristic habitats. It can grow in media with glucose as the sole organic constituent. Wild-type *E. coli* has no growth factor requirements, and metabolically it can transform glucose into all of the macromolecular components that make up the cell. The bacterium can grow in the presence or absence of O_2 [15]. Under anaerobic conditions it will grow by means of fermentation, producing characteristic "mixed acids and gas" as end products. However, it can also grow by means of anaerobic respiration, since it is able to utilize NO_3, NO_2 or fumarate as final electron acceptors for respiratory electron transport processes. In part, this adapts *E. coli* to its intestinal (anaerobic) and its extra-intestinal (aerobic or anaerobic) habitats [15]. *Escherichia coli* are a normal inhabitant of the vagina and are seen to colonize Up to 20% of pregnant women [16]. These colonizing isolates can sometimes cause complications during pregnancy or can be transmitted to the neonate leading to neonatal infection [15,17]. Ability to colonize and cause infections has been

attributed to the presence of several virulence genes in this organism [18]. Vaginal colonization with *E. coli* is reported as a risk for very low birth weight delivery and other perinatal complications [19].

Studies from worldwide have reported isolation of drug resistant *E. coli* among vaginal isolates of pregnant women [13,20,21]. Transmission of these resistant strains to the neonate can prove fatal in whom early detection is challenging and treatment options are limited. Outbreaks in neonatal wards and adverse outcome due to drug resistant *E. coli* infection have been reported [22,23]. Thus identification and elimination of these resistant strains at the maternal level can have an impact on the reduction of fatal outcome in neonates especially in developing countries where the neonatal mortality rate is high [24]. In this context a pilot study has been done to determine the resistance pattern and plasmid profile of *E. coli* colonizing the vagina of asymptomatic pregnant women. Pregnancy causes numerous changes in the woman's body [13]. Hormonal and mechanical changes increase the risk of urinary stasis and vesicoureteral reflux. These changes, along with an already short urethra (approximately 3-4 cm in females) and difficulty with hygiene due to a distended pregnant belly, increase the frequency of urinary tract infections (UTIs) in pregnant women. Indeed, UTIs are among the most common bacterial infections during pregnancy. In general, pregnant patients are considered immunocompromised UTI hosts because of the physiologic changes associated with pregnancy [15]. These changes increase the risk of serious infectious complications from symptomatic and asymptomatic urinary infections even in healthy pregnant women [19].

Pathogenesis of *Escherichia coli*

Escherichia coli typically colonize the gastrointestinal tract of human infants within a few hours after birth. Usually, *E. coli* and its human host coexist in good health and with mutual benefit for decades. These com-mensal *E. coli* strains rarely cause disease except in immunocompromised hosts. Despite the enormous body of literature on the genetics and physiology of this species, the mechanism whereby *E. coli* assures this auspicious symbiosis in the colon is poorly characterized [25]. One interesting hypothesis suggests that *E. coli* might exploit its ability to utilize gluconate in the colon more efficiently than other resident species, thereby

allowing it to occupy a highly specific metabolic niche. However, there are several highly adapted *E. coli* clones that have acquired specific virulence attributes, which confers an increased ability to adapt to new niches and allows them to cause a broad spectrum of disease. These virulence attributes are frequently encoded on genetic elements that can be mobilized into different strains to create novel combinations of virulence factors, or on genetic elements that might once have been mobile, but have now evolved to become 'locked' into the genome. Only the most successful combinations of virulence factors have persisted to become specific 'PATHOTYPES' of *E. coli* that are capable of causing disease in healthy individuals [25].   Three general clinical syndromes can result from infection with one of these pathotypes: enteric/diarrhoeal disease, urinary tract infections (UTIs) and sepsis/meningitis. Among the intestinal pathogens there are six well-described categories: enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli*, (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli*. (EIEC) [25].

Diversity of *Escherichia coli*

Escherichia coli encompass an enormous population of bacteria that exhibit a very high degree of both genetic and phenotypic diversity. Genome sequencing of a large number of isolates of *E. coli* and related bacteria shows that a taxonomic reclassification would be desirable. However, this has not been done, largely due to its medical importance and *E. coli* remains one of the most diverse bacterial species: only 20% of the genome is common to all strains. A strain is a sub-group within the species that has unique characteristics that distinguish it from other strains. These differences are often detectable only at the molecular level; however, they may result in changes to the physiology or lifecycle of the bacterium. For example, a strain may gain pathogenic capacity, the ability to use a unique carbon source, the ability to take upon a particular ecological niche or the ability to resist antimicrobial agents. Different strains of *E. coli* are often host-specific, making it possible to determine the source of fecal contamination in environmental samples. For example, knowing which *E. coli* strains are present in a water sample allows researchers to make assumptions about whether the contamination originated from a human, another mammal or a bird [26].

Serotypes *Escherichia coli*

A common subdivision system of *E. coli*, but not based on evolutionary relatedness, is by serotype, which is based on major surface antigens (O antigen: part of lipopolysaccharide layer; H: flagellin; K antigen: capsule), e.g. O157:H7). It is however common to cite only the serogroup, i.e. the O-antigen [27]. At present about 190 serogroups are known. The common laboratory strain has a mutation that prevents the formation of an O-antigen and is thus non-typeable. Enteric *E. coli* (EC) are classified on the basis of serological characteristics and virulence properties [28]:

- Enterotoxigenic *E. coli* (ETEC)

- Enteropathogenic *E. coli* (EPEC)

- Enteroinvasive *E. coli* (EIEC)

- Enterohemorrhagic *E. coli* (EHEC)

- Enteroaggregative *E. coli* (EAEC) [5].

Multiple drug pumps in *E. Coli*

Bacterial multidrug resistance is a result of multidrug efflux pumps, which are inner membrane transporters that export several drugs from within to the outside of bacterial cells (MDR). Experimental systems where the pump protein is overexpressed and substrates are identified by enhanced resistance to a panel of drugs have been used in part to identify multidrug efflux pump systems and their substrates. The development of efflux pump inhibitors is essential for combating infectious disorders brought on by bacteria that are multidrug resistant since a single multidrug efflux pump can export numerous drugs [29,30]. The multidrug efflux pump AcrB in *Escherichia coli* is one example.

In *E. coli*, multidrug pumps work together to lower the intracellular concentration of medicines, which serves as the first line of defense against antimicrobials. A number of transporter proteins that are found in the periplasm and membrane of bacterial cells remove various extraneous substrates from bacterial cells, such as antimicrobials, organic solvents, poisonous heavy metals, etc., to form this protective barrier. Several multidrug transporters are encoded on the *Escherichia coli* chromosome. Despite serving as a barrier against antibacterial medicines, it is still unclear how exactly these transporters function physiologically. Under physiological circumstances, the bacterium *E. coli* creates the tryptophan metabolite indole.

Escherichia coli has more than seven efflux systems that can

export antibiotics with unrelated structures; these MDR pump systems let bacteria develop inherent resistance to harmful substances such antibiotics, antiseptics, detergents, and dyes. They are of interest because of their unidentified physiological roles [31], potential roles in clinical resistance [32,33], potential use as antibacterial targets [32,33], and potential role in cell-based antibacterial drug discovery [34]. The biological studies of *E. coli* have discovered seven distinct proton-dependent MDR pump systems: AcrAB-TolC [35], EmrAB [36], MdfA [37], TehA [38], EmrE [39], AcrEF [40,41]. Analyses of comparable amino acid sequences have helped to identify others. According to a compilation by Paulsen et al. [42], all belong to one of three different families: the main facilitator superfamily (MFS), the resistance nodulation-cell division (RND), and the small multidrug resistance (SMR).

Numerous amphiphilic compounds can be expelled by bacteria over the cell membrane in an energy-dependent way. These compounds, which normally inhibit bacterial development [29,42], include basic dyes, antibiotics, and detergents. As a result of this efflux, bacteria have developed resistance to these substances. The fundamental physiological activities of these efflux proteins are not fully understood, despite the fact that they are implicated in intrinsic antibiotic and antiseptic resistance in bacteria [42-44]. Heavy metals, hydrophobic organic compounds, detergents found in the environment, antibiotics produced by other microorganisms, and other hazardous substances could all be warded off by Efflux proteins [42,45]. An *Escherichia coli* multidrug efflux pump called AcrAB has recently been described by Thanassi et al. [46] as pumping different bile acids out of the bacteria [46]. In an environment like the mammalian gastrointestinal tract, which is rich in bile salts, this activity may be crucial for bacterial survival. These efflux proteins, which are chromosomally encoded, may also be able to evacuate potentially dangerous metabolic wastes generated by the bacteria.

Staphylococcus aureus

Staphylococcus aureus is a common bacterium which is carried on the skin and/or in the nose of approximately 20 to 40% of otherwise healthy individuals [28]. As long as this organism remains on the surface, it generally causes no harm. However, under the right circumstances, it can cause a broad range of infections ranging from mild skin conditions such as boils or furuncles to potentially life-threatening

infections involving the blood, lungs, or other organs and tissues in the body. Prior to the start of the modern era of antibiotics in the early 1940s, *Staphylococcus aureus* was fully susceptible to penicillin. However, soon after the introduction and widespread use of penicillin into clinical practice, this organism quickly adapted to become penicillin-resistant. Although initially it was found only in the hospital setting, it eventually moved into the community to become extremely common. The mechanism of this resistance is mediated through the production of an enzyme known as beta-lactamase which is capable of destroying the active sight of penicillin and thus rendering it ineffective. The rapid emergence and spread of beta-lactamase producing *Staphylococcus aureus* lead to the development of semi-synthetic penicillins, such as methicillin, cloxacillin, oxacillin, and nafcillin which are not destroyed by this beta-lactamase enzyme. These drugs have become known as "beta-lactamase stable penicillins". Within a year of their introduction in 1960, *Staphylococcus aureus* once again quickly adapted and developed a new mechanism of resistance to these agents. By producing a new or altered target site that no longer allowed these agents to bind to them, the emergence of Methicillin Resistant *Staphylococcus aureus* (MRSA) was borne [28].

Penicillinase

Penicillinase is a specific type of β-lactamase, showing specificity for penicillins, again by hydrolysing the beta-lactam ring [9]. Molecular weights of the various penicillinases tend to cluster [47]. Penicillinase was the first β-lactamase to be identified, was first isolated by Abraham and Chain in 1940 from Gram-negative *Escherichia coli* even before penicillin entered clinical use, but penicillinase production quickly spread to bacteria that previously did not produce it or produced it only rarely [48,49]. Penicillinase-resistant beta-lactams such as methicillin were developed, but there is now widespread resistance to even these antibiotics [50].

Quinolones

The quinolones are a family of synthetic broad-spectrum antibacterial drugs. The first generation of the quinolones begins with the introduction of nalidixic acid in 1962 for treatment of urinary tract infections in humans. Nalidixic acid prevent bacterial DNA from unwinding and duplicating [51]. Quinolones, in comparison to other antibiotic classes, have among the highest risk of causing colonization with MRSA and Clostridium difficile. The majority of quinolones

in clinical use belong to the subset fluoroquinolones, which have a fluorine atom attached to the central ring system, typically at the 6-position or C-7 position such as ciprofloxacin [51].

Quinolones inhibit the topoisomerase II ligase domain, leaving the two nuclease domains intact. This modification, coupled with the constant action of the topoisomerase II in the bacterial cell, leads to DNA fragmentation via the nucleic activity of the intact enzyme domains [52]. Recent evidence has shown eukaryotic topoisomerase II is also a target for a variety of quinolone-based drugs. Thus far, most of the compounds that show high activity against the eukaryotic type II enzyme contain aromatic substituents at their C-7 positions [52]. Quinolones can enter cells easily via porins and, therefore, are often used to treat intracellular pathogens such as Legionella pneumophila and Mycoplasma pneumoniae. For many Gram-negative bacteria, DNA gyrase is the target, whereas topoisomerase IV is the target for many Gram-positive bacteria. Some compounds in this class have been shown to inhibit the synthesis of mitochondrial DNA [52,53].

CONCLUSION

Beta-lactamase are enzymes produced by some bacteria that provide resistance to beta-lactam antibiotics like penicillins, cephamycins, and carbapenems (ertapenem), although carbapenems are relatively resistant to beta-lactamase. Beta-lactamase provides antibiotic resistance by breaking the antibiotics' structure.

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It is therefore recommended as follows:

a) It is therefore necessary to place surveillance of antibiotics in our Society to avoid increase antibiotics resistance producing organisms.

b) Routine monitoring of antibiotic resistance to provides data for antibiotic therapy and resistance control with information will directly affect selection of empiric therapy for urinary tract infection in pregnant women is recommended to promote good antenatal management in the health sector.

c) The awareness of the existence of ESBL is recommended to initializes indication for the need for proper use of antibiotics and spread of multi drug resistance bacterial strains within these hospital and communities.

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