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CRITICAL REVIEW

Carbapenem-resistant *Enterobacteriaceae*: Resistance mechanisms and alternative strategies

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ABSTRACT
Carbapenem-resistant *Enterobacteriaceae* (CRE) are the alarming outcome of an ongoing biological arms-race between humans and infectious bacteria. Once considered a last resort class of antibiotics, carbapenems are now effectively evaded by CREs through porin downregulation and efflux pump upregulation mechanisms. Together, these bacterial systems work to reduce the toxicity of carbapenems by preventing their entrance into the cell. More important is the production of diverse classes of carbapenemases, enzymes which effectively inactivate carbapenems by hydrolyzing the β-lactam ring of β-lactam antibiotics. The various mechanisms of these carbapenemases compound the problem of infection treatment to a point where drugs are not being developed fast enough to counter the rapid evolution of resistance. In the face of this antibiotic crisis, it is important to focus attention on prevention and detection strategies in addition to treatment techniques.

INTRODUCTION

The introduction of antibiotics has contributed to the vast eradication of infectious organisms. Since then, the boundaries of antibiotic properties have been manipulated to counter emerging infections and introduce novel bactericidal mechanisms. Penicillin and streptomycin work via bacterial cell wall synthesis inhibition and protein synthesis inhibition, respectively.[1] These are early examples of mechanisms among the enormous repertoire of antibiotics at our disposal.[1] Despite the antimicrobial evolution, targeting specific bacteria is becoming increasingly arduous due to a co-evolutionary arms race: the rise of antibiotic resistance. In recent years, the emergence of a specific group of Gram-negative bacteria, carbapenem-resistant *Enterobacteriaceae* (CRE), symbolizes the necessity of alternative modes of eradication. These bacteria are resistant to carbapenems, which are considered the most potent group of β-lactam antibiotics. Thus, CREs represent an intricate and significant problem in the clinical setting.[2] The following review will comment on the mechanisms of β-lactam antibiotics and resistance in CREs, with the aim of contextualizing the importance of optimized prevention and detection strategies.

REVIEW FINDINGS

β-Lactam antibiotics

β-lactam antibiotics are a class of antibiotics that can be categorized into four structural subdivisions: penams, cephems, monobactams, and carbapenems, the last of which is considered the most potent form.[3] The potency of carbapenems is attributed to their distinctive molecular configuration, consisting of a pyrroline ring fused to a β-lactam.[4] This configuration provides the antibiotic class with stability against a broad spectrum of β-lactamases in addition to reduced susceptibility to resistance elements, such as degradation and inactivation.[4] Most β-lactam antibiotics function by interfering with the transpeptidation step of peptidoglycan biosynthesis.[5] Peptidoglycan is the principle component of the cell wall in nearly all bacteria and serves as the stress-bearing layer and cell shape determinant. The efficacy of β-lactam antibiotics is attributed to the fact that they structurally mimic specific precursor peptides involved in peptidoglycan cross-linking. This property allows β-lactam antibiotics to irreversibly bind the transpeptidase domain of penicillin-binding proteins (PBP), preventing the cross-linking of peptidoglycan.[6] The structural integrity of the bacterial cell wall is thus compromised, leading to cell death.[5,6]

Among other β-lactam antibiotics, carbapenems, once considered a last resort antibiotic, are now being rendered impotent by emerging strains of CREs.[7] The mechanisms of resistance are becoming increasingly diverse and difficult to circumvent as they are adapted to specific selective pressures, such as drug-mediated removal.[6,8]

CREs: Mechanisms of resistance, carbapenemase classes

Selective pressures that threaten the survival of bacterial species initiate evolutionary mechanisms which drive the development of antibiotic resistance.[9] Such evolutionary mechanisms include horizontal gene transfer (HGT), which allows for the acquisition of external DNA, and genetic mutations.[9] Typically, bacteria acquire resistance genes through three HGT mechanisms: transformation (the process of spontaneous DNA uptake), transduction (the process of DNA transfer between a bacteriophage-infected bacterium and an uninfected bacterium),

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and conjugation (the pili-mediated transfer of DNA between adjacent bacteria).[10] The incorporation of external DNA into the bacterial genome or plasmid may provide resistance to bacteria that did not previously possess it. In contrast, most genetic mutations result in antibiotic target site modification, upregulated efflux mechanisms, downregulated influx pathways, and metabolic pathway alterations.[9] For most bacteria that have acquired resistance through either genetic mutations or HGT, the major mechanistic pathways remain: enzymatic inactivation or degradation of the antibiotic, alteration of the antibiotic target, and modification of membrane permeability.[11]

In CREs, carbapenem efficacy is either drastically reduced or rendered completely ineffective through three main modes of resistance: efflux pump upregulation, decreased outer membrane permeability via porin downregulation, and carbapenemase production.[6] Efflux pumps redistribute a successfully penetrated antibiotic back into the extracellular environment.[12] In contrast, porins increase permeability; thus, through porin expression downregulation, CREs can effectively prevent antibiotics from penetrating their cellular envelopes.[13] Finally, carbapenemases provide CREs with the strongest mode of resistance. These are specific and unique classes of β-lactamases —enzymes which hydrolyze β-lactams— that selectively target and inactivate carbapenems.[14] The general mechanism of most β-lactamase enzymes involves the hydrolysis of the β-lactam ring. Without the β-lactam ring, β-lactam antibiotics are unable to bind to PBP and disrupt cross-linking between peptidoglycan polymers.[15] As a result, bacteria continue to thrive unaffected in their environment.

Mechanistic variations between different classes of carbapenemases pose a challenge in targeting them.[16] These carbapenemases are classified as either serine-carbapenemases (classes A, C, D) or metallo-β-lactamases (class B). In general, class C is considered to have weak activity for carbapenems, rendering its clinical significance uncertain.[6]

Class A serine carbapenemases utilize Ser70 in their active sites to facilitate the hydrolysis of β-lactam rings.[17] Previous research suggests that this mechanism proceeds in two steps: acylation and deacylation.[17] In the acylation step, Ser70 of the carbapenemase acts as a nucleophile and attacks the amide bond of the β-lactam. This interaction creates an acyl-enzyme complex, where Ser70 remains covalently modified by the drug. The deacylation step begins with the activation of a deacylating water molecule by conserved glutamate and tyrosine residues. The deacylating water subsequently hydrolyzes the acyl-enzyme complex and releases the inactivated open-ring form of the β-lactam and the free enzyme.[17] The intricacy of class A carbapenemases is increased by structural variations between their different forms. For instance, class A carbapenemase SME-1 exhibits shorter positional distances between Ser70 and Glu166 compared to other variants, facilitating more effective hydrolysis.[18] This phenomenon depicts the level of complexity that exists within specific classes in addition to inter-class variation.

Class D carbapenemases facilitate ring hydrolysis similar to class A in that both utilize acylation and deacylation steps.[19] In both, the acylation steps involve a catalytic serine residue to produce an acyl-enzyme intermediate. In contrast to class A, the deacylation step involves the use of a carboxylated lysine residue to activate a deacylating water molecule, rather than by glutamate and tyrosine.[19] Similar to class A, class D also has intra-class variation within its diverse group of enzymes. For instance, the Oxa 24/40 variant consists of a hydrophobic bridge which facilitates easier carbapenem active site hydroxyethyl group entry.[19]

Class B metallo-β-lactamases contain a Zn^{2+} ion cofactor(s) in their active site, necessary for β-lactam ring hydrolysis.[12] Class B1 and B3 enzymes contain two such Zn^{2+} ions, whereas class B2 enzymes contain only one.[12] Previous studies have indicated that the binding of another Zn^{2+} ion on class B2 enzymes would instead decrease their activity.[12,20] These enzymes use different mechanistic pathways compared to those from class A and D because their catalytic activity does not

involve the hydroxyl group of a serine residue. Instead, the Zn^{2+} ion cofactor(s) activates a water molecule, which serves directly as the catalytic nucleophile to attack the β-lactam ring. Several other amino acids, along with Zn^{2+} ions and an additional water molecule, participate in various stabilizing interactions to complete ring hydrolysis.[12]

The implications of these findings suggest that the co-evolution of bacterial resistance is becoming increasingly complex; alternative strategies which do not introduce significant selective pressures may be required. The implementation of such strategies may improve patient outcomes and overall public health.

FUTURE DIRECTIONS

Current antibiotic treatments are becoming increasingly diverse in order to target different bacterial functions. Further research is also being conducted on quorum-sensing methods and anti-bacterial vaccines to combat the emerging antibiotic resistance crisis.[21,22] This evolutionary arms race may, however, be a losing battle if prevention and detection strategies are not optimized. The diversity of CRE resistance pathways, in tandem with complicated variations in carbapenemase mechanisms, represents the near loss of an extremely potent antibiotic that was once considered a last resort in the clinical setting. It is of greater concern that the rate of resistance evolution is outpacing the development of new drugs, as more complex and lengthy research needs to be performed to target evolved mechanisms.[7] Therefore, it is paramount to consider preventative and screening measures for CREs.[7] Implementing proper hygiene practices and limiting antibiotics to only required usage remain crucial.[7] Clinicians should be wary of prescribing antibiotics as unnecessary usage increases selective pressures and facilitates resistance development.[23] Several studies have found that antibiotics are over-prescribed in hospital settings, with some identifying 50% of antibiotic prescriptions as being inappropriate.[24,25] The apt and accurate detection of CREs is also critical in informing clinical decisions involving antibiotics. Numerous measurement methods are available for further investigation, including phenotypic methods such as the Modified Hodge Test and the Carbapenem Inactivation Method, as well as genotypic methods such as Metagenome Sequencing and Conventional Polymerase Chain Reactions.[2] Such measures should be implemented in a timely fashion in order to inform clinical decisions.

CONCLUSION

Antibiotic resistance is a growing worldwide threat with catastrophic health implications. The problem also brings with it complicated social and economic ramifications which necessitate immediate attention. By 2050, it is estimated that drug resistance will result in 10 million global deaths per year, with 315 000 of those being in North America alone, and a loss of over 100 trillion CAD from the global GDP.[26,27] In the face of this global and rapid biological arms race, it is important to consider strategies beyond treatment techniques, such as those of prevention and screening, and work towards a more sustainable global solution.

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