Review

Prevalence and Characterization of Multidrug Resistant Enteric Bacterial

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Accepted 21 November, 2014

Antibiotic resistance in bacteria has been a concern in the medical field for almost as long as antibiotics have been available. The last several decades have seen marked increases in antibiotic resistance, leading to the discovery of multidrug-resistant (MDR) bacteria, which can be resistant to several antibiotics. MDR bacteria are a major problem in the healthcare industry, creating numerous challenges such as reduced treatment options, increased mortality rates, longer hospital stays, and increased costs. The increasing dissemination of resistance genes is believed to be the result of horizontal gene transfer via mobile genetic elements, including plasmids and transposons. However, most of the studies investigating the role of integrons in the dissemination of antibiotic resistance utilized bacterial samples from environmental sources or hospitalized patients. Far fewer studies have examined the role of integrons in the propagation of multidrug-resistance in bacteria from the lower intestinal tract of healthy individuals. The purpose of this study was to determine whether or not integrons play a significant role in the proliferation of multidrug-resistance in enteric bacteria isolated from healthy, non-hospitalized adults. Attempts were also made to identify the gene cassettes and organization of cassettes within the identified integrons. Subsequent sequencing and nucleotide BLAST searches led to the identification of eight different gene cassettes organized in six unique arrays.

Key words: Antibiotic resistance, bacteria, multidrug-resistant (MDR).

INTRODUCTION

In the early years of antibiotic treatment, many scientists and doctors believed that infectious disease had been triumphed once and for all (Davies, 2007). Less than a decade after the first antibiotics were introduced in medicine, evidence of bacterial strains resistant to those antibiotics began to surface (Davies, 2007). Shortly thereafter, scientist’s uncovered evidence that bacteria were not only capable of developing resistance to one antibiotic, but to multiple antibiotics that were also transferable to sensitive strains (Davies, 2007). The rise of multidrug-resistant (MDR) bacteria is a result of unscrupulous antibiotic use in medicine and agriculture over the last several decades (Davies, 2007). Today, MDR bacteria provide numerous challenges and problems for healthcare providers, including increases in hospital-acquired infections, reduced treatment options, higher morbidity and mortality rates, and healthcare cost increases due to longer hospital stays (Ducel, 2002).

Perhaps the most widely publicized strain of MDR bacteria is the much-feared Gram-positive methicillin resistant Staphylococcus aureus (MRSA) (Davies and Davies, 2010). However, less well-publicized MDR Gram negative bacteria are also capable of causing serious, difficult to treat infections. The Antimicrobial Availability Task Force, established by the Infectious Diseases Society of America, identified several particularly problematic pathogens, one of which included extended-spectrum beta-lactamase (ESβL) producing Enterobacteriaceae (e.g. Escherichia coli and Klebsiella pneumoniae) (Talbot,2006). ESβLs are enzymes produced by bacteria that confer resistance to multiple antibiotic classes, namely cephalosporins, penicillins, monobactams, and beta-lactamases(Talbot,2006). Over 500 different ESβLs have been identified, the most common belonging to the CTX and CMY gene families (Talbot, 2006). Infections caused by ESβL producers
Transposons are genetic elements that may be inserted into plasmids, transposons can also carry resistance genes. Mobile elements; they are frequently associated with strong antibiotic resistance. Bacteria can rapidly acquire new genes that make them immune to various antibiotics. Conjugation. The recipient bacterium acquires all genes from one bacterium to another in a process called horizontal gene transfer (Carattoli, 2001; Davies, 2007; Davies, J. and Davies, D., 2010. Roe, 2003), where genetic information is passed directly from one bacterium to another. Horizontal transfer of antibiotic resistance genes occurs primarily through two different genetic elements: plasmids and transposons. Plasmids are small, circular, extrachromosomal DNA molecules that may contain resistance genes. Plasmids can be transferred via a pilus from one bacterium to another in a process called conjugation. The recipient bacterium acquires all genes present on the plasmid, including resistance genes. Like plasmids, transposons can also carry resistance genes. Transposons are genetic elements that may be inserted into and excised from chromosomes and plasmids. Through sharing of DNA via these two mechanisms, bacteria can rapidly acquire new genes that make them immune to various antibiotics.

A third group of genetic elements that have been strongly implicated in the emergence of MDR bacteria are called integrons. While integrons themselves are not mobile elements: they are frequently associated with transposons and plasmids. Plasmid-integrated transposons carrying antibiotic resistance genes can be transferred to other bacteria through conjugation (Iyer, 2013). Integrons are capture-and-expression genetic elements that facilitate site-specific recombination of promoter-less gene cassettes into a site that allows for the transcription of all genetic material contained in the cassettes. They consist of three main components located in the 5’ conserved region: an integrase gene (int), a recombination site (att), and an active promoter (Magiorakos et al., 2013). The integrase recognizes a conserved, 59-base element (actually varies in length from 57-141 bases), which is found on resistance gene cassettes. Upon recognition of this conserved element, the integrase facilitates the integration of the cassette into the integron at the att site, just downstream of the active promoter. Any cassettes that are integrated downstream of the promoter are then free to be transcribed; they may also be rearranged or excised via the integrase, and new promoter-less resistance genes can be integrated. Thus, integrons are essentially genetic elements capable of integrating and expressing various rearrangeable antibiotic resistance gene cassettes that can be readily mobilized into neighboring bacteria.

**Nomenclature of Multidrug-resistant Enteric Isolates**

Antibiotic-resistant organisms residing as part of a person’s intestinal flora, whether they are pathogenic strains or not, may act as a reservoir for resistance genes that can be transferred to other bacteria (Marshall, 2009). Bacteria are able to transfer resistance genes horizontally to one another through various mechanisms. The emergence of MDR bacteria is the result of horizontal gene transfer (Carattoli, 2001; Davies, 2007; Davies, J. and Davies, D., 2010. Roe, 2003), where genetic information is passed directly from one bacterium to another. Horizontal transfer of antibiotic resistance genes occurs primarily through two different genetic elements: plasmids and transposons. Plasmids are small, circular, extrachromosomal DNA molecules that may contain resistance genes. Plasmids can be transferred via a pilus from one bacterium to another in a process called conjugation. The recipient bacterium acquires all genes present on the plasmid, including resistance genes. Like plasmids, transposons can also carry resistance genes. Transposons are genetic elements that may be inserted into and excised from chromosomes and plasmids. Through sharing of DNA via these two mechanisms, bacteria can rapidly acquire new genes that make them immune to various antibiotics.

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**DISCUSSION**

**PCR Detection of Class 1 and Class 2 Integrasens**

At least three classes of integrons have been identified, which are distinguished primarily by the integrase gene. Genes contained within the 3’ conserved region also vary between the three classes of integrons. Class 1 and class 2 integrons are the most prevalent and best studied (Fluit, 2004). Class 3 integrons appear to be far less common, and therefore less implicated in the spread of multidrug-resistance. Class 3 integrons have been found in Serratia marcescens, Klebsiella pneumoniae as well as Delftia species. Class 1 integrons, on the other hand, have been found in many Gram-negative Enterobacteria, including species of Escherichia, Klebsiella, Pseudomonas, Enterobacter, Salmonella, Proteus, Serratia, Citrobacter, and Shigella. Integrons are known to contain highly conserved regions at the 5’ end (which encodes the integrase gene) as well as the 3’ end, downstream of integrated gene cassettes. The 3’ conserved region of class 1 integrons consists of the qacA1 and sul1 genes, which confer resistance to quaternary ammonium compounds and sulfonamides, respectively. Class 2 integrons appear to be less widespread, although they have been identified in several genera of bacteria, such as Shigella, Salmonella, and Acinetobacter, as well as Escherichia, Morganella, and Aeromonas (35). Integrons are believed to play a considerable role in the dissemination of antibiotic resistance genes within Gram-negative bacteria. A group of researchers recently created a database, called the
Repository of Antibiotic resistance Cassettes (RAC), which contains over 300 different promoter-less gene cassettes. Several of these antibiotic resistance gene cassettes are frequently seen integrated into both class 1 and class 2 integrons, including those granting resistance to aminoglycosides, cephalosporins, chloramphenicol, penicillins, and trimethoprim (Carattoli, 2001).

The association between antibiotic resistance and integrons has been well documented. Integrons have been shown to be particularly prevalent in many clinical isolates of Gram-negative enteric bacteria. Integron frequencies in clinical samples as high as 88% and as low as 13% have been found, though more common frequencies fall in the range of 20%-60% (Peirano et al., 2006, White et al., 2006). There have also been numerous studies investigating the prevalence of integrons in bacteria isolated from sources other than humans. Such sources include wastewater treatment plants irrigation sediments and animals. Far fewer studies have been conducted to investigate the prevalence of multidrug-resistance in bacteria obtained from healthy, non-hospitalized individuals. Studies that include commensal bacteria obtained from humans often include clinical isolates or a combination of animal and human derived specimens (Mazel, 2000). One study that investigated integrons in a mixed sample set of animal, commensal human, and clinical human isolates did find that MDR was associated with the presence of integrons regardless of origin, indicating that a positive correlation between MDR and commensal human isolates had been established. Another study investigated the transfer of antibiotic resistance genes among nonpathogenic Bacteroides within the human colon, but no attempt was made to identify the presence of integrons or investigate their possible role (Shoemaker, 2001).

Through an IRB-approved exemption, a collection of antibiotic-resistant enteric bacteria from healthy CSUS students was accumulated over the course of five years. Multidrug-resistance was observed in several of the enteric isolates. I hypothesized, based on previous research, that the prevalence of class 1 and class 2 integrons would be significantly greater in multidrug-resistant enteric bacteria comprising normal flora of healthy adults than in isolates with low or no resistances. Few studies have attempted to examine the prevalence or role of integrons in the propagation of MDR bacteria that exist as part of the normal human intestinal flora. By determining the prevalence of integrons within the drug-resistant samples collected, some insight may be gained into the role of integrons in the dissemination and maintenance of multidrug-resistance factors in the community.

CONCLUSION

Integrons play a significant role in the proliferation of multidrug-resistance in enteric bacteria isolated from healthy, non-hospitalized adults. Attempts were also made to identify the gene cassettes and organization of cassettes within the identified integrons. The study also identifies the gene cassettes and organization of cassettes within the identified integrons. Subsequent sequencing and nucleotide BLAST searches led to the identification of eight different gene cassettes organized in six unique arrays.

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