

Therapeutic Potential of Chitosan Nanoparticles as Antibiotic Delivery System: Challenges to Treat Multiple Drug Resistance

Mohammed M. Safhi¹, S. M. Sivakumar², Aamena Jabeen², Foziyah Zakir², Farah Islam², U. S. Bagul², Seyda Sanobar³, Tarique Anwer¹, Rahimullah Siddiqui¹, Mohammad Abdul Hakeem Siddiqui¹, Mohamed Eltyep Elmobark², B. B. Barik², Mohammad Firoz Alam¹

¹Department of Pharmacology and Toxicology, College of Pharmacy, Jazan University, Jazan, KSA,

²Department of Pharmaceutics, Division of Pharmaceutical Biotechnology, College of Pharmacy, Jazan University, Jazan, KSA, ³Department of Pharmacognosy, College of Pharmacy, Jazan University, Jazan, KSA

Abstract

Antibiotic treatment for bacterial infection is becoming a big challenge due to the expression of a resistant factor which is a major hurdle in curing the infectious diseases, especially nosocomial infections. Globally, multiple drug resistance has emerged as a major public health problem. Nanotechnology is the most unique technology that can efficiently deliver the antibiotics to the cell site. In this review, we are focusing on the use of chitosan nanoparticles as an antibiotic delivery system to improve the therapeutic efficiency of antibiotics and to overcome the antibiotic resistance. We conclude that chitosan nanoparticles are promising therapeutic carriers to deliver antibiotics and can overcome efflux mechanism of bacteria.

Key words: Antibiotic resistance, Chitosan polymer, Efflux pumps, Nanoparticles

INTRODUCTION

The bacterial resistance to antibiotics is the major threat to treat infectious diseases. Bacterial genes are expressed based on environmental factors leading to utilization of various biochemical pathways to escape the lethal action of antibiotics. Resistance may be due to altered cell membrane permeability, diminished transport across the inner membrane, alternation due to mutation or enzyme modification. In general, multiple antibiotics resistance in Gram-negative bacteria often starts with relatively limited outer membrane permeability to many antibacterials coupled with the expression of R factor to give resistance. For past two decades, vancomycin resistance has been observed against *Enterococcus* infection.^[1,2] Collis and Hall, 1995^[3] reported that the structural genes that mediate antibiotic resistance often are closely linked and may exist in tandem along the bacterial chromosome or plasmid. Genetic analysis of DNA sequences adjacent to antibiotic-resistance genes revealed

that unique integration units often exist near promoter sites. These integration elements called integrons function as “hot spots” for site-specific recombination events between largely non-homologous sequences of DNA. Integrons also serve as expression cassettes for antibiotic-resistance genes, in which an efficient promoter site is provided in close proximity to the 5' end of the newly inserted DNA sequence.^[4] The frequency of transcription of integrated cassettes of antibiotic-resistance genes depends on the proximity of the gene to the promoter at the 5' upstream end of the integron.^[5] The level of expression of a resistance gene diminishes as the distance between the promoter and the specific antibiotic-resistance gene cassette increases.

Address for correspondence:

S. M. Sivakumar, Department of Pharmaceutics, Division of Pharmaceutical Biotechnology, College of Pharmacy, Jazan University, Jazan, KSA.
E-mail: drsmsivakumar@gmail.com

Received: 09-04-2016

Revised: 20-04-2016

Accepted: 25-04-2016

Many researchers had reported bacterial resistance against various antibacterials with respect to organisms such as *Streptococcus pyogenes*, *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Neisseria gonorrhoeae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enterica*, *Pseudomonas aeruginosa*, and *Vibrio cholerae*.^[6-10] Bacterial resistance to antibiotics/antibacterials has been studied since the discovery of penicillin G and sulfonamides in 1940.^[11] Antibiotic resistance has opened the way to usage of a higher dosage of antibiotics/antibacterials to combat infection, especially hospital-acquired infections worldwide. This leads to higher toxicity and increased mortality.^[12] In the year 2000, the World Health Organization warned world governments about the emergence of resistance to drugs. If the world government failed to control infections by avoiding drug resistance, the entire population could be wiped out.^[13] Many researchers across the world had attempted to resolve the resistance problem by discovering new antibiotics or by changing the structure of the moiety. However, many antibiotics are ascending in more spectrum of activity, but unfortunately, there is no assurance that the bacteria will not develop resistance against newer moieties. In connection to this, nanotechnology is the most unique technology that can provide suitable tools for effective targeted delivery of drugs into specific cells. Some of the recent studies focused that nanoparticles encapsulated antibiotics commonly known as nanobiotics restore their ability by increasing the concentration of antibiotics at the site of bacterium-antibiotic interaction and facilitate binding of antibiotics to bacteria. Targeting the infection is greatly achieved by nanoparticles carrier system since the size of nanoparticle in nanoscale enables the transport through extracellular and intracellular biological barriers. Moreover, nanoparticles can be easily phagocytosed by antigen presenting cells especially dendritic cells and thereby increases the antibiotics concentration in sustained manner with lesser dose and can overcome the multiple drug resistance problem.

Chitosan is one of the most used polysaccharides in the design of drug delivery strategies for various drugs and biologicals.^[14] The interaction between protonated amino groups of chitosan with cell membranes results in a reversible structural reorganization of proteins associated tight junctions facilitating their opening. Because of this property, chitosan has been used for the preparation of mucoadhesive formulations for drug targeting systems and for formulations that enhance the macromolecular therapeutics. Therefore, the best hope for the future is the development of nanobiotics to avoid drug toxicity as well as multiple drug resistance.

MULTIPLE ANTIBIOTIC RESISTANCE – A THREAT

Globally, resistance to antibiotics has emerged as a therapeutic challenge, especially for nosocomial infections since hospitalized patients are at higher risk of infection

due to various reasons.^[15] The irrational utilization of the antibiotics is the main drive for resistance development across the world, especially in developing countries due to lack of knowledge and understanding on the proper usages of antibiotics. Moreover, the prescription pattern by physicians due to uncertain diagnosis is also an important factor to be considered for the development of antibiotic resistance.^[15] Apart from this, the utilization of antibiotics rationally in veterinary medicine for disease prevention, agriculture, aquaculture, and horticulture also counts as a major contributing factor for developing antibiotic resistance. Resistance to antibiotics has been recorded since 1940.^[16] In 1947, Barber^[17] reported about the penicillin resistance against *S. aureus* due to enzyme penicillinase. Bacteria may develop resistance after a particular antibiotic treatment due to mutation of genes^[18] or acquiring of foreign genes from other organisms by horizontal transfer.^[19,20] Multiple drug resistance is due to expression of resistant plasmid or transposon genes. The resistant plasmid is very small, <10 kb in size that encodes resistant genes, whereas conjugative plasmid is larger than R plasmid >30 kb in size or even more, which encode the conjugation functions causing the cell to cell coupling especially in Gram-negative bacteria.^[21] However, gene transfers in Gram-positive bacteria reflect a different mechanisms of the cell to cell coupling. The size of conjugative plasmids in Gram-positive bacteria is smaller than Gram-negative bacteria, which requires less genetic information.^[22] Transposons are mobile genes (Jumping genes) that can exist on plasmid or integrate into host chromosome that express the proteins such as transposase or recombinase.^[23] Integrons contains a collection of genes called gene cassettes that imparts the recombination function. They can easily integrate into the stable regions of other targeted DNA. Plasmid-encoded antibiotic resistance is notable in antibiotic therapy using cephalosporins, fluoroquinolones, and aminoglycosides. Enzymes expressed by bacteria can also modify the effect of antibacterial drugs. e.g., β lactamase is involved in the degradation of β lactam antibiotics. Macrolides, and aminoglycoside modifying proteins lead to chemical transformations. Aminoglycoside modifying enzymes are usually present on the integrons and other mobile elements. Genes responsible for acetyltransferase enzyme is present on integrons of *P. aeruginosa* also imparts resistance to sulfonamides.^[24] Acetyltransferase is inactivate chloramphenicol; Flavin-dependent monooxygenase is inactivate tetracyclines and its derivatives.^[25] Resistance to fluoroquinolones is due to mutations with drug target enzymes DNA gyrase and topoisomerase.^[26] Mutations in gene specifying dihydropteroate synthetase decrease the enzyme affinity for sulfonamides and overexpression of the enzyme leads to reduced affinity of trimethoprim. The use and misuse of antibiotics in the treatment of not only humans but also in veterinary, agriculture, and aquaculture have led to antibiotic resistance.^[26]

The development of antibiotic resistance is due to the expression of resistant genes either during treatment or due

to natural environment factor. However, newer molecular techniques have been developed for detecting resistant factors such as PCR and microarrays, which serve as major diagnostic tools.^[27-29] Using these techniques, several novel antibiotic-resistance genes have been identified including β lactams,^[30,31] aminoglycosides,^[32,33] and bleomycin.^[34] Metagenomics is an approach that is used to identify the genetic potential of microbes by detecting presence or absence of genes or genetic variations. Regulatory proteins expressed by genes of chromosomes control multiple drug resistance by acting as activators or repressors of transcription characterized in *E. coli*, *Bacillus subtilis*, *S. aureus*, *N. gonorrhoeae*, and other *Enteric bacteria*.^[35]

MOLECULAR MECHANISM OF MULTIPLE DRUG RESISTANCE

The expression of resistance against antibiotics is due to the expression of resistant gene that code to develop altered substrate to which antimicrobial agents bind leading to inactivated antibacterial activity.^[36] The antibacterial drug resistance occurs through various mechanisms, among which the most common multidrug resistance mechanism is drug efflux pumps. The antibacterial drug efflux system has been grouped into five:

1. Major facilitator super (MFS) family
2. The ATP-binding cassette (ABC) family
3. The resistance nodulation division (RND) family
4. Small multiple drug resistant (SMR) family
5. Drug/metabolite transporters.

In general, Gram-negative bacteria are typically resistant to antimicrobials than the Gram-positive bacteria. In Gram-negative, RND transporters express the activity in conjugation with protein associated with periplasmic membrane called periplasmic membrane functional protein, and a protein factor called outer membrane factor.^[37] The most important efflux pump is ABC transporter. They are principle transmembrane transporters responsible for import and export of various substances such as sugars, amino acids, various ions, and drugs.^[38] RND efflux system has been recognized in number of Gram-negative organisms such as *E. coli*, *Salmonella typhimurium*, *P. aeruginosa*, *S. enterica*, *Mycobacterium*

species, and *N. gonorrhoeae* [Table 1]. Due to this efflux mechanism, many Gram-negative bacteria have reduced the uptake of the drug.^[39] Gram-negative bacteria have an outer membrane composed of peptidoglycan which surrounds the periplasmic space that surrounds inner membrane. The outer membrane provides a barrier against the uptake of drugs especially hydrophobic drugs, which is not present in Gram-positive bacteria.^[40] This is one of the reasons why Gram-negative bacteria are less susceptible to antimicrobials than Gram-positive bacteria. *P. aeruginosa* is a Gram-negative bacterium that has an outer membrane, surrounding periplasmic space that surrounds inner membrane. The drug efflux pump has inner membrane proton (H⁺)/drug antiporter that bound to a linker protein of periplasmic space that bound to outer membrane protein and the organism becomes resistant due to the mutation process that leads to overexpression of efflux protein (In *E. coli* there are nine transmembrane protein gradient pumps that have been recognized as a cause for multiple drug resistance. However, the proton-dependent efflux pumps are grouped into the three families MFS family, SMR family, and resistant nodulation cell division family (RND). Among these three, the most explored efflux pumps is RND pump called AcrAB/TolC. Due to the mutation of Acr gene, the expressed protein causes antibiotic efflux which leads to antibiotic resistance. Multiple drug resistance is also due to the expression of a resistant gene that produces an altered substrate where the antibacterials attach that leads to inactivation of antibacterial activity.^[41] Bacteria can also produce biofilms that prevent their contact with antibacterials, and thus resistance develops.^[42] Therefore, transfusion of drugs through cell membrane has become an important target to overcome multiple drug resistance. Biodegradable polymeric nanoparticles can easily diffuse the cell membrane by escaping the membrane bound protein efflux pumps, escapes from alternation of drug moiety and safely deliver the drugs inside the cell.

CHITOSAN NANOPARTICLE AS ANTIBIOTIC DELIVERY SYSTEM

The current era of pharmaceutical research is focusing on nanoparticle drug delivery system, especially on polymeric nanoparticles. Drug encapsulation and delivery through

Table 1: Selected bacterial efflux pumps and antibiotics associated with drug resistance

Organism	Efflux pumps	Antibiotic associated
<i>Pseudomonas aeruginosa</i>	MexAB oprM	Tetracyclines, chloramphenicol, fluoroquinolones
<i>Escherichia coli</i>	AcrAB TolC	Chloramphenicol, lipophilic β lactams, fluoroquinolones, tetracycline, rifampicin, novobiocin, fusidic acid, nalidixic acid
<i>Salmonella enterica</i>	Ade ABC	Chloramphenicol, nalidixic acid, tetracyclines
<i>Streptococcus pneumoniae</i>	Pmr A	Fluoroquinolone
<i>Mycobacterium species</i>	Rv 1634, Rv 1258c, Rv 2686c, Rv 2687c	Fluoroquinolone, rifampicin
<i>Neisseria gonorrhoeae</i>	MtrCDE	Penicillin's, macrolides, tetracyclines

nanoparticles has attained increasing interest as it is smaller in size and easily target the cells. Throughout the last decade diverse bacterial resistance mechanisms such as decreased uptake, augmented efflux of antibiotic/antibacterial from bacterial cell, formation of biofilms to keep away from contact with antibiotics have been identified that lead to failure in treatment. Therefore, instead of developing novel antibiotics or antibacterial drugs to combat infections, which are cost effective, it is advisable to design novel drug delivery strategies using polymeric nanotechnology that facilitates effective delivery of drug to the infection site by reducing the dosage, toxicity, and thereby improving therapeutic efficacy. Biodegradable polymeric nanoparticles formulated for drugs, vaccines, and plasmid DNA are being explored by various researchers. A variety of synthetic and natural polymers have been investigated for sustained release of drugs and vaccines. The development of modern technique has lead to the selection of appropriate carrier for drugs to release them in sustained or controlled manner. Chitosan is a copolymer of glucosamine and N-acetylglucosamine, obtained by deacetylation of chitin, a natural abundantly available polymer (e.g., in crustaceans). The ratio between glucosamine and N-acetyl glucosamine is referred as degree of deacetylation.^[43] Chitosan is a weak base, insoluble in water and organic solvents but soluble in dilute acid solutions (pH < 6.5) and gets precipitated with alkaline solution.

Chitosan and its derivatives have been reported as potential carriers for drug delivery systems.^[44] Structurally, chitosan can be modified to get many derivatives. Due to non-toxic, biocompatible, biodegradable, high charge density and mucoadhesion properties, chitosan has a broad range of applications in pharmaceuticals with various aspects have been published by Sivakumar *et al.*, 2014 and 2013.^[44,45] The main advantage of chitosan polymer is it being, a polycationic polymer can easily attach with anionic microbial cells [Figures 1 and 2] and thereby increasing its permeability.^[46] Some of the earlier reports show that chitosan exhibits antimicrobial activity by binding with DNA and thereby preventing transcription processes. Recently, Safhi *et al.*, 2014^[47] has reported the efficacy of penicillin G loaded chitosan nanoparticles against various bacteria. In their study, maximum activity was found in *S. pyogenes* followed by *B. subtilis* and *S. aureus*. Their study suggested that chitosan nanopenicillin showed better activity than the conventional penicillin.

CONCLUSION

The success of controlled delivery systems is to maintain the minimum drug concentration at the specific site of infection which improves therapeutic compatibility. Therefore, we conclude that chitosan nanoparticles are promising therapeutic carriers to deliver antibacterials in naive form and it can overcome mechanism of bacteria, gene mutations, etc.

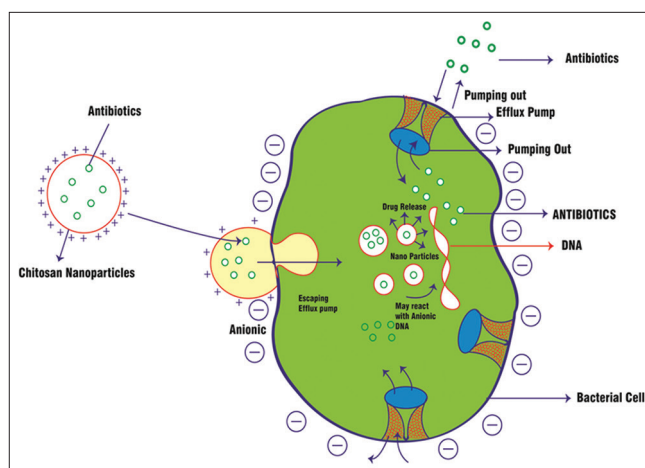


Figure 1: An overview of nanoparticle mode of antibiotic delivery inside the bacterial cell

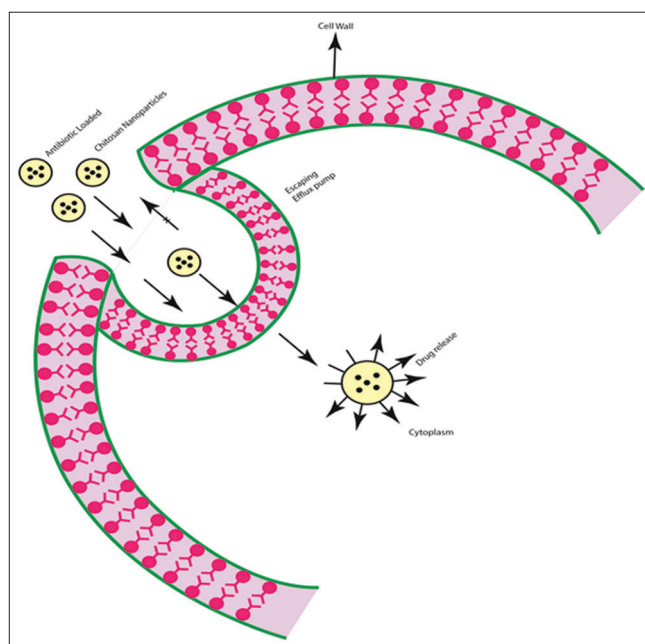


Figure 2: Antibiotics loaded chitosan nanoparticles escaping efflux pump at cell membrane level and enter into the bacterial cell

Thus, the efficacy of antibacterial drugs can be increased and multiple drug resistance problems can be easily combated.

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Source of Support: Nil. **Conflict of Interest:** None declared.