An Overview of New Delhi Metallo-Beta Lactamase-1 and Extended Spectrum Beta Lactamase Producing Bacteria: Need for an Alternate

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Abstract

Resistance to antibiotics is increasing dangerously worldwide. The emergence of antibiotic resistant bacteria is no more a local problem and calls for global action. It has become a leading challenge in infectious diseases management. The effectiveness of current drugs is restricted by emergence of multi-drug resistant bacterial strains and they have become the major reason for treatment failure of infections. Antimicrobial agents were initially highly successful in treating infections; however, their unsound use leads to rise in antimicrobial resistance frighteningly, especially in the developing countries. With the emergence of New Delhi Metallo-beta lactamase-1 and Extended Spectrum Beta Lactamase producing bacteria the clinicians are left with a very limited choice of treating the common infections. The recent situation calls for an urgent need for search of alternatives like herbal medicine which has shown potential in treating various other ailments. Because herbs are plants, they are often perceived as "natural" and therefore safe. Development of plant based compounds and combinational therapies using compounds may provide safer options for the community. Research should focus on scrutinizing compounds from plants for a target and bioactivity based drug discovery. However, the safety of using most herbs with drugs is not well established. Some herbs are known to interact with pharmaceutical drugs, although most of this information comes from case reports rather than systematic investigations. Because many herbs contain pharmacologically active compounds, some herbs may cause side effects through excessive biological effects. Unfortunately, the true frequency of side effects for most herbs is not known because most have not been tested in large clinical trials and because surveillance systems are much less extensive than those in place for pharmaceutical products. There is also a need for a strict regulations and policy in place for the use of herbal drugs or compounds of herbal origin in treatment of various common bacterial infections.

Keywords: Multi-drug resistance, NDM-1, ESBL, Herbal drugs

Introduction

Overuse and misuse of antibiotics has led to rapid evolution of antibiotic resistance across the globe [1]. Consumption of antibiotics globally capita has increased by 70% within the last decade [2]. Antimicrobial resistance has evolved since the beginning of the antibiotic era causing a menace in 21st century. A worldwide antimicrobial consumption in animals was 63 151 (± 1560) tons in 2010 and projected to rise by 67% by 2030. This increase in antimicrobial resistance has resulted increase in the cost of treating infectious diseases [3]. Hospital infections, high prevalence of infectious diseases, inadequate public health systems, and easy access to inexpensive antibiotics without prescription together increase the resistance [4].

The microorganisms eventually become Multi-Drug Resistant (MDR) forms on continuous use of various antibiotics and become "superbugs". The "superbugs" are microbes with increased resistance due to several mutations that contain high levels of resistance to the many diseases [5]. Among the resistant pathogens the fast evolving gram negative bacteria include *Acinetobacter sp., Citrobacter sp., Enterobacter sp., Klebsiella sp., P. aeruginosa, Escherichia coli, Nerisseria gonorrhoeae, Salmonella spp, Enterococcus faecium, Streptococcus pneumonia, Haemophilus influenza* and *Shigella spp* including the Mycobacterium tuberculosis [6]. The modes of resistance in such bacterial species include altered target sites, specialized efflux pumps, DNA mutations that develop alternate metabolic pathways and production of enzymes that degrade antibiotics [7-9]. Resistance by the production of beta lactamase enzymes (ESBLs and Carbapenemases) has now become a threat around the world and WHO lists such bacteria in a critical priority category for the discovery of new drugs [6].

Carbapenems were the last line of treatment for most Extended Spectrum Beta Lactamase (ESBL) producing resistant organisms, however after the emergence of New Delhi Metallo-beta lactamase 1 (NDM-1) a recently evolved MBL has shown resistance to most classes of antibiotics including carbapenem except tigecycline, and colistin [10]. Demise for antibacterial drug discovery has been created after the discovery of a few NDM producing strains and gram-negative bacteria that are also resistant to colistin [11-13]. In this scenario, crisis for new antibiotics and untreatable infections prevail. Thus a significant impact in the discovery of alternative drugs from plant sources is a necessity as we are now in the "last resort" of antibiotics [14].

Prevalence of ESBL and NDM-1

Plasmid mediated beta lactamases that include metallo beta lactamses (MBLs) and Extended spectrum beta lactamases (ESBL) genes spread rapidly to different bacterial species, therefore, rapid modification of therapy, discovery of alternative drugs and initiation of infection control to prevent their dissemination are necessary [15].The

extended spectrum beta-lactamases (ESBLs) including the PER, VEB-1, BES-1, TEM and SHV-type beta-lactamases have the ability to hydrolyze penicillins, first, second and the third generation cephalosporins [16]. The ESBL producing bacteria are commonly also found to co-exist with Amp C, and CTX-M genes that makes such MDRs difficult to treat [17]. The carbapenems are still the first choice of treatment for serious infections associated with gram negative bacteria that includes β- lactamase and ESBL-producing organisms [18,19]. ESBL-producing organisms were first detected in Europe and eventually reported in the other parts of the world [16,20]. Although the exact global prevalence of ESBL producing organisms is not known, certain studies in the Indian subcontinent have found nearly 50% prevalence [21,22].

Resistance to carbapenem is however observed recurrently among gram-negative bacteria that involves production of MBLs. New Delhi Metallo-beta lactamase 1 (NDM-1) first discovered in a Swedish patient in 2008, had an alarming impact globally and bought the discovery of antibiotics to a halt. NDM producers were found to co-exist with blaOXA genes [23,24], rmtC [25], rmtF [26] genes that brings down the treatment options. This threat further increased when the emergence of colistin resistant NDM producing bacteria was reported [13]. The novel NDM-1 not highly identical to other MBLs and is closely associated to VIM-1/VIM-2, with around 32.4% identity [27]. The major dissemination of NDM-1 producers were found in the Asian continent distributed mostly in China and India with around 58.15% abundance [28].The ESBLs and NDM-1 enzyme vary structurally the former having a serine within the active site while the latter two zinc ions within its active site respectively [29,30]. This alters the treatment regimens and strategies which in turn increases the demand for varied development perspectives for new antimicrobials [18,28,31].

Detection of antibiotic resistance for ESBL and NDM-1 producers

Detection of resistance for these rapidly evolving microorganisms is an essential factor for the specific treatments. The assays such as double disk synergy test (DDST) or the Etest are used to determine the production of these beta-lactam enzymes [32]. DNA sequencing of the amplified PCR products from the isolates that carries these genes are purified using the PCR DNA purification kit and subjected to automated DNA sequencing. Susceptibility to various classes of antibiotics can be determined by disc diffusion method as prescribed by Clinical Laboratory Standard Institute (CLSI) guidelines is one of the most commonly used techniques to detect resistance. Apart from general tests, few specific tests are developed for ESBL and NDM-1. Presence of ESBL microorganisms are tested using Vitek2 ESBL test panel which analyses the synergy between ceftazidime, cefotaxime or cefepime and clavulanic acid. Few other tests are using EbSA ESBL screening agar, ChromID ESBL screening agar plates, and MIC-strip for ESBL and MicroScan panels [33,16].

A TaqMan chemistry has been developed to detect NDM-1 carbapenemase genes from bacterial isolates and directly from stool samples using a quantitative real-time PCR (qPCR) assay [34]. CHROMagar KPC culture medium was proposed for an outbreak of NDM-1 for early detection of NDM-1 producers for any hospitalized patients on a worldwide scale and for at-risk patients in areas of where it may prevent outbreaks of NDM-1 producing infections [35]. Loop Mediated Isothermal Amplification (LAMP), for detecting blaNDM-1 gene in NDM-1 positive bacteria was developed which was a rapid

non-culturable method [36,37]. A electrochemical biosensor was developed to detect blaNDM-1 gene encoding the NDM-1 enzyme, using label-free electrochemical impedance spectroscopy (EIS). In this EIS assay, they have used a blaNDM-specific PNA probe that was designed by applying a strategy that combines in-silico probe design and DNA microarray (fluorescence-based) validation with electrochemical testing using gold electrodes [38]. The membrane attachment of β-lactamases includes detection of carbapenemase activity by the MHT and a new method developed showed that this problem can be rectified by the addition of Triton X-100, called the Triton Hodge test, which allows detection of membrane-bound carbapenemases [39]. DiaPlexC™ CRE Detection Kit was made to detect 4 kinds of CRE genes, IMP, VIM, KPC and NDM using the Multiplex PCR. This can detect multiple specific target genes in a single PCR. NDM-1 ELISA kits are highly sensitive, and specific diagnostic assay for detection of blaNDM-1 based on cross priming amplification (CPA) was developed. The results showed that the CPA could detect as low as 2 copies of plasmid DNA, being more sensitive than PCR as well as LAMP [40].

Treatment options

Extensive efforts are taken to design multiple strategies to combat multidrug resistance and so far very little have been achieved. ESBLs frequently associated with genes encoding resistance to aminoglycosides and trimethioprim/sulfamethoxazole along with cephalosporin resistance. Clavulanic acid reduces the minimum inhibitory concentrations (MICs) of cephalosporins when treated against the ESBLs. Although carbapenems are not used as a first line of treatment for the ESBL-producing organisms they are mostly used against serious infections, due to their stability to withstand hydrolysis by ESBLs [41]. NDM-1 is resistant to all the antibiotics including β-lactam antibiotics only a few promising compounds have been reported so far to combact its resistance. Aspergillomarasmine-A (AMA), a natural compound from *Aspergillus versicolor*, was reported to inhibit NDM-1 activity by extracting zinc ions from its active site. AMA was able to fully restore the antibacterial activity of meropenem against NDM-1 harboring bacteria from *Enterobacteriaceae, Acinetobacter spp.* and *Pseudomonas spp.* [42]. Ebselen was shown to covalently bind with the Cys residue at the active site of NDM-1, thereby, offering a new inhibition mechanism with a potential broad spectrum inhibitory activity [43]. D-captopril binds to the active site of recombinant NDM-1 with high binding affinity, and inhibits its enzymatic activity [44]. Two thiol-modifying compounds, p-chloromecuribenzoate (p-CMB) and nitroprusside were also reported to inhibit the activity of NDM-1. p-CMB inhibited NDM-1 by modifying the Cys residue in the active-site of the enzyme. However, mutating this residue to Asp did not affect the enzyme activity and it has become resistant to the inhibitor [45]. Thirteen synthetic diaryl-substituted azolylthioacetamides were found to inhibit the activity of the NDM-1 enzyme isolated from *Aeromonas veroni* [46]. Combinatorial Genetics En Masse (CombiGEM) strategy was employed to identify transcription factor combinations to enhance killing of bacteria by β-lactam drugs against resistant *Escherichia coli* NDM-1 strains [47]. CRISPR-Cas for genome-editing was also used to target and modify antibiotic resistant genes [48]. Khan et al. (2017) [49] has discovered five non-beta-lactam inhibitors through virtual screening and confirmed efficacy of these molecules using in vitro cell assays.

Need for alternate treatment

This global emergence of multi-drug resistant (MDR) bacteria is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure [50] and urges for an urgent call for search of alternate resources for combating this growing menace. Plants have been used for health and medical purposes for several thousands of years. WHO also stressed on reduction of antibiotic usage by applying alternative strategies. The number of higher plant species on earth is about 250,000. It is estimated that 35,000 to 70,000 species have, at one time or another, been used in some cultures for medicinal purposes. A majority of the world's population in developing countries still relies on herbal medicines to meet its health needs. The use of medicinal plant extracts and studying their pharmacological properties can lead to the development of modern therapeutic agents. Medicinal plants are important sources for pharmaceutical manufacturing. Medicinal plants and herbal medicines account for a significant percentage of the pharmaceutical market. For example, in China, medicinal plants and their products had a 33.1% share of the pharmaceutical market in 1995. In Malaysia, the market for traditional medicine is estimated at about 1 billion Malaysia rinngit annually [51]. The plant compounds such as flavanoid family and anthocyanins could give more potential microbial-inhibitory compounds that selectively influence intestinal bacteria species. Plant based drugs have a long history of use and better patient tolerance. They are renewable sources, cheap, inexpensive and locally available in many developing countries [52]. The simplest bioactive phytochemicals have a single substituted phenolic ring called simple phenols and phenolic acids. Structures of quinones contain two ketone substitutions in their aromatic rings. Phenolic structures that contain one carbonyl group are flavonoids or flavones and flavonol consists of addition of one 3-hydroxyl group. Plants fighting against microbial infections synthesize such compounds. Tannins are a group of polymeric phenolic substances found in bark, wood, leaves, fruits, and roots of almost every plant [53]. Phenolics can act as metal chelators that increase their catalytic activity or reduce metal ions, thus increasing their ability to act as antioxidants [54]. Flavonoids are reported to possess several properties, including oestrogenic, anti-inflammatory, antimicrobial, enzyme inhibition, vascular, antiallergic and antitumour activity [55]. Maddox et al. [56] have shown that different subgroups of flavonoid compounds, including flavan-3-ol (catechin), flavanone (naringenin), flavonol glycoside (rutin), and flavonol aglycone (quercetin) showed antibacterial activity against *Xylella fastidiosa*. The results of antimicrobial activity of 17 phenolic compounds showed different bacterial species demonstrate different activity profiles against phenolics. Differences in sensitivity to one flavonoid were observed with various strains of the same bacterial species. The berry extracts tested showed inhibition mostly of Gram-negative but not Gram-positive bacteria [57]. Diterpenes, such as ent-3-hydroxy-beyer-15-ene-2-one and diosphenol 2 isolated from *Spirostachys africana* showed activity against many Gram positive and Gram negative microorganisms. The compound 3-acetyl aleuritolic acid was found to inhibit various gram negative pathogenic microorganisms including *Salmonella typhi*, *V. cholera* and *S. dysentery* [58]. Monoterpenes like menthol, thymol, and linalyl acetate inhibited both the gram-positive *Staphylococcus aureus* and the gram-negative *Escherichia coli* by their membrane-damaging activity, which was shown by measuring the release of trapped carboxyfluorescein from large unilamellar vesicles [59]. The lectins act on cells in many ways, such as mitogenic stimulation, agglutination and killing [60, 61]. These peptides inhibit the microbial membrane by interfering in the formation of ion channels or competitively inhibit the attachment of microbial proteins to the receptors on membrane [62,63]. The most commonly used alkaloids medically are morphine, ephedrine, quinine, piperine etc. Their bioactivity and challenging structures led to various modes of activity profile. The alkaloids showed inhibitory effects against various gram negative and gram-positive microorganisms and also against many fungal species [64]. Numerous natural products from plants such as angusticornin B, bartericin A, 6-hydroxy-7-methoxyluteolin, madreporanone, stachytriol have exhibited antibacterial activity [65-67]. PYN6 isolated from this plant has shown activity against major classes of infectious bacteria including MRSA [68]. Terpenoids from Ocimum spp. [69,70], berberine from Berberis vulgaris [71], tannin and terpenoids from Eucalyptus globules [72], β-Resercyclic acid an organic acid from Cannabis sativa [73] and flavonoid like quercetin and phenolic acid like caffeic acids from Camellia sinensis [74] are some antibacterial compounds isolated from plants. Phenolics such as flavonoids and tannins act in membrane disruption, enzyme inactivation and metal ion complexation. Terpenoids act in membrane disruption and alkaloids intercalate to cell wall or DNA [64]. 1,4-Naphthalenedione from *Holoptelea integrifolia* [54], ethyl gallate from *Caesalpinia spinosa* [55] and corilagin from *Arctostaphylos uva-ursi* [75] were found to be active against beta-lactamase producing *Staphylococcus aureus*. Caffeic acid, ellagic acid, epigallocatechin-3-gallate, quercetin isolated from *Punica granatum* were effective against beta- lactamase producing [76]. Metalo beta lactamase producing *Stenotrophomonas maltophilia* was inhibited by galangin a plant derived compound [77]. Although many plant extracts can contain toxic compounds that can give adverse effects if wrongly consumed [78-80]. Several research studies reported on consumption of alternative and complementary medicine (CAM) with allopathic medicine has not showed any adverse side effects. Most people also preferred to take CAM along with allopathic medicines as the effect of allopathic medicines were not satisfactory. Such combinational treatments with CAM and allopathic medicines requires further studies to assess, improvise and develop correct combinations to increase treatment options [81,82]. Also, isolation of specific compounds makes the compound specific to target with lesser side effects instead of whole plant extracts. Although more research is required to assess long term toxic effects of plant compounds more beneficial results are associated with the plant compounds if taken in correct doses [80,83]. It is also indicated that plants gave compounds that show positive synergism when combined with antibiotics [84-86]. Saklani et al. [87] reports several plant derived anti-infective and anticancer compounds and a total of 91 plant derived compounds were in the clinical trials in 2007. Koehn et al. [88] have reported that natural products from plant sources have higher number of chiral centers, oxygen atoms, hydrogen bond donors and acceptors. Such nature of chemical compounds benefits and enables to modify and develop analogues by changing the side chains to increase specificity and make modify the compound according to the target sites. This nature also facilitates deletion of functional groups that can be used to reduce the toxicity of the compound [89]. This approach is based on a hypothesis that the active compounds isolated from plants source could be safer than the ones extracted from plant species which shows minimal history of human use.

Previously, screening was done to isolate natural compounds against *Escherichia coli* harbouring NDM-1 from microbial sources [42], however the plant kingdom being the main source of drugs, remained unexplored. Plants have diverse chemical structure which can have several modes of action to inhibit MDR bacteria but until limited

number of alternative compounds from plant sources have been reported. Chandar et al (2017) [90] screened 240 plant extracts which could be used further to develop formulations followed by isolation of active compounds. This study is the first large-scale screening for checking antibacterial activity of plant extracts against NDM-1 producing pathogenic organisms. In the study, ethanol extract from the leaves of 240 taxonomically diverse medicinal plant species representing 183 genera and 75 families were screened for antibacterial activity against NDM-1. The use of colistin was abandoned during 1970s due to nephrotoxicity [91] but re-emerged as lifesaving antibiotic to combat multidrug- resistant NDM-1 bacteria [10] and in the due course colistin resistance has also evolved [11-13]. Interestingly, reduction in MIC of colistin, meropenem and tetracycline was observed in all combinations of plant extracts [90]. Thus this combinational therapy could vastly help to reduce the antibiotic usage (colistin and carbapenems) and consumption in the long run which will in turn decrease the antibiotic resistance. Thus, alternative new drug development is in a continuous need for transformation of medicine in order to combat antimicrobial resistance. The safety of using most herbs with drugs is not well established. Some herbs are known to interact with pharmaceutical drugs, although most of this information comes from case reports rather than systematic investigations [92]. Because many herbs contain pharmacologically active compounds, some herbs may cause side effects through excessive biological effects. Unfortunately, the true frequency of side effects for most herbs is not known because most have not been tested in large clinical trials and because surveillance systems are much less extensive than those in place for pharmaceutical products [93]. This also calls for a strict regulation and policy to be formulated for use of herbal drugs in treating various common infections.

Conclusion

In the menace of antibiotic resistance, it is important to monitor the consumption of antibiotics and determine treatment ways to reduce the usage. Therefore, development of plant based compounds and combinational therapies using compounds may provide safer options for the community. Further research should focus on scrutinizing compounds from plants for a target and bioactivity based drug discovery. The government should also play an important role in promoting and supporting research in herbal medicine to evade the impending danger of no treatment alternate for deadly pathogens in our near future.

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