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Efficient approaches for combating the antibiotic resistance of gram-negative bacterial pathogens against conventional drugs

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Abstract

Antimicrobial resistance is a well-known concept and an expanding field of study since it is the main cause of major health problems worldwide. It acts as one of the most dangerous threats confronting humans today. Three bacteria are on the World Health Organization's list and are classified as crucial to the greatest level of concern. All three resistant bacteria belong to the gram-negative group. Extended-spectrum lactamases, AmpC enzymes, and carbapenemase are among the effective mechanisms that have led to the widespread spread of gram-negative bacterial resistance. Antibiotic resistance has evolved across several bacterial strains. Gram-negative bacteria (GNBB) are studied to have a significant resistance pattern against antibiotics than Gram-positive bacteria because of their peculiar structure; they can cause major morbidity as well as mortality all over the world. Several tactics for modulating the spread of antibiotic resistivity in the gram-negative bacterial population have been studied and proposed, including the use of novel chemical compounds that have a different mode of action than antibiotics and the development of target molecules that act as receptors to kill resistant bacteria. Through the deactivation of certain resistance mechanisms, some chemical molecules and procedures have exhibited efficacy. Another interesting trend is the development of some compounds that are naturally synthesized and have antibacterial properties, like bacteriophages, DCAP, metal-dependent anti-bacterial agents, Odilo-rhabdins (ODLs), and quorum-sensing (QS) inhibitor. The development of contemporary therapies for Gram-negative bacteria that surpass multiple drugs is the predominant focus of this study, and novel combinations of therapeutic targets are being explored.

Keywords: Gram-negative bacteria; Antibiotic resistance; Multidrug drug resistance; Antimicrobial; Alternative therapies

1. Introduction

Throughout history, a variety of natural products have been used to treat plethora of illnesses. Quinine, for example, is found in cinchona malaria [1]. Numerous antibacterial medications have been developed since Fleming's discovery of penicillin in 1929, and these have had a significant impact on both human health and the mortality rate [2]. Widespread misuse of antibiotics has led to the emergence of antibiotic resistance (Fig. 1) [3]. Unfortunately, medical stores in developing nations, in particular, sell common antibiotics without a prescription. As a result, antibiotic resistance is spreading. There is an urgent need to impart education about the impact of such practices on public health [4].

The discovery of novel medicines or adaption of a drug in order to combat with the bacterial multi-drug resistance capacity, the global effort has now reached unprecedented proportions [5]. When a bacterial cell shows its efficiency to evade the effectiveness of antibiotics through certain distinct pathways such as by neutralising the effect of antibiotics or through pumping them out from the cell or by altering the structural conformation, it results in the inhibition of the target drug's attachment to bacterial cell surface [6]. This eventually develops the bacterial resistance capacity towards multiple drug molecules [7]. Antibiotic resistance mechanisms can be classified into certain distinct groups such as intrinsic resistance, acquired resistance, inactivation of drug, modification of target drug molecule, limiting the intake of drug molecule by body, active efflux of drug etc [8]. In case of intrinsic resistance bacterial cell undergo a structural modification, process or change their structural components [9]. On the other hand, in case of acquired resistance a new gene is acquired [10]. It is basically transfer of a newly synthesized resistant gene from resistance bacteria to sensitive bacteria and making other

bacteria also resistance [11]. Furthermore because of genetic alteration, the synthesis of protein, that results in alternative component synthesis and receptors which do not get recognised by the antibiotic happens [12]. This finally leads to genomic DNA or horizontal gene transfer between bacterial species through different process such as transformation, transduction or conjugation [13].

Antimicrobial resistance is a major issue in hospitals and clinical set up [10]. WHO produced a list of germs in 2017 (Fig. 2.) for which new medicines are urgently required, categorising them as critical, high, or medium priority [1].
This has been evidently found that gram-negative bacteria are main cause of human disease and infection especially among immune-compromised individuals [2]. This may happen due to multi drug resistance (MDR), nosocomial or hospital acquired infections because of economic burden on health care system. These infections are induced by pathogenic gram-negative bacilli (GNB) such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa and Stenotrophomonas maltophilia and Enterobacteriaceae* and non-fermenting GNB [3]. Nosocomial infections includes catheter related bloodstream infections, ventilator associated pneumonia, ICU acquired sepsis, urinary tract infections, etc [4] [5].

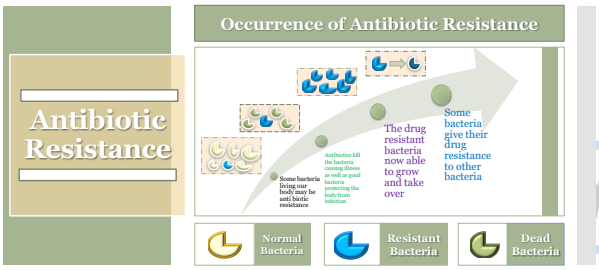

Fig. 1. The mechanism behind the increase in antibiotic-resistant bacteria is the evolutionary stress that these medications cause. This tendency can be accelerated by improper antibiotic usage, which is extremely risky for healthcare in the future.

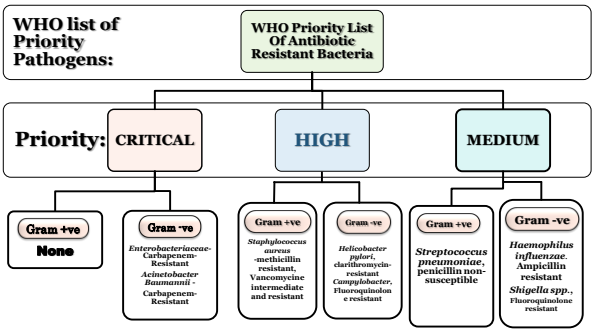

Fig. 2. Priority infections identified by World Health Organization (WHO) are divided into three groups based on their level of antibiotic resistance: critical, high, and medium.

2. Gram negative bacterial resistance to anti-bacterial drugs

The morphology of Gram positive bacteria differs from that of Gram-negative bacteria [6]. Gram-negative bacterial morphology includes following characteristics: lipid membrane that surrounds the body [8], an appropriately thin layer of peptidoglycan (2 to 3 manometers) [14], teichoic acids are generally absent [15], flagella or pili may be present [12]. The primary differentiation is found in the outer lipid membrane [16]. It is tough to penetrate this outer membrane made of lipid bi-layer, which provides added protection to gram-negative bacteria. [17]. Gram-negative bacteria seem to be more difficult to kill because of this special characteristic [18]. As a result, both the gram-positive as well as the gram-negative bacteria require different treatments [19]. Gram-positive bacteria also can causes infections in humans, although gram-negative bacteria are much more difficult to eradicate [20]. Antimicrobials are required for the treatment of several species that cause disease [21].
The outer membrane (Fig. 3.) has been identified as the primary cause of promoting resistance to gram negative bacteria against common antibiotics used to treat infections caused by them, which includes quinolones, β-lactam, colistin and others [22]. In several researches and development process of antibiotics and its mode of action study, it has been observed that the antibiotics have to cross outer membrane of the bacterial cell wall to reach the target site [23]. The pathways are different among different groups of antibiotics based on their polarity [24]. For example, active or passive diffusion pathway are use by hydrophobic drugs to cross the membrane, whereas, porins are used by hydrophilic antibiotics to get inside the bacterial cell [1]. Vancomycin is one of the greatest exceptions in this regard as it fails to pass through membrane and does not follow any either pathway mentioned above [2]. As mentioned earlier, that the primary cause of resistance among gram-negative bacteria occurs due to the change the structural conformation of the cell wall [3]. This is done either through modification of structural components or by altering the hydrophobic characteristics of membrane proteins and lipid [4]. Mutations in porins and other variables also lead in development of resistance [5]. The significant characteristics and lacking of specialised layers in the cell wall structure, make gram-negative bacteria more susceptible to develop resistance capacity to several drugs or antibiotics [6]. In GNB the antimicrobial resistance mechanism (Fig. 4.) occurs due to the presence or expression of antibiotic-inactivating enzymes [8]. This also includes some of the non-enzymatic routes that emerge from increased intrinsic resistance due to chromosomal gene changes [7]. The chromosomal gene changes include alterations in target site, permeability, increased expression of antibiotic-inactivation enzymes and over expression of efflux pumps etc [14]. It can also be acquired through

mobile genetic elements transfer that carries the resistant gene such as β-lactamases and aminotransferases producing plasmid [24].

A study on resistance to third-generation cephalosporins among *Enterobacteriaceae* has shown that the resistance is now greater than 10% whereas the susceptibility for carbapenem is only 2-7% [15]. This has taken place because of the rapid spreading of bacteria that produce extended-spectrum-lactamase (ESBL) [25]. Nosocomial infections caused by *klebsiella pneumoniae* the resistant towards carbapenems surpasses 25% rate, although *P. aeruginosa* resistance rates span from 20 to 40%, and *A. baumannii* carbapenem resistance rates range from 40 to 70% [26] . In this review the more significant resistant Gram-negative bacteria on such a global scale and its several treatment methods in order to combat with the resistance capacity has been successfully discussed here.

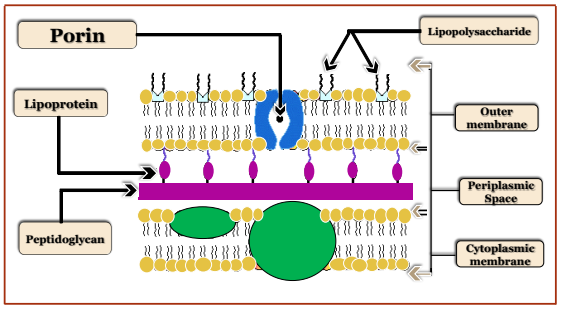

Fig. 3. A diagram that describes the structure of Gram-negative bacterial cell wall

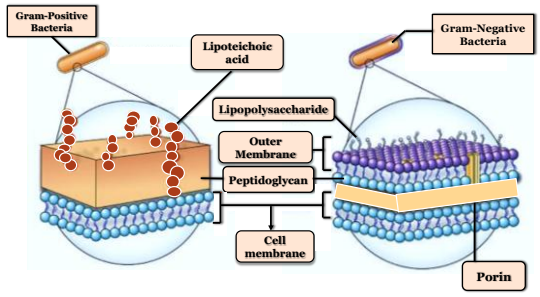

Fig. 4. A diagrammatic representation of both Gram Positive and Gram-Negative bacterial Cell Wall

3. Resistant Gram-Negative Bacteria

MDR bacteria are gram-negative bacterial species those are resistance to numerous antibiotics [27, 28]. They are capable to induce bacterial infections, which constitute a major and quickly rising risk to hospitalised patients, particularly those in critical care units [28]. Infections produced by MDR strains (Fig. 5.) are responsible for higher mortality, morbidity and long stay in the hospital. These germs not only endanger worldwide public health, but they also place a huge strain on healthcare systems [29]. Because of the scarcity of treatment choices and the scarcity of newly discovered antimicrobial drugs, these bacteria constitute a significant hazard to public health [30]. MDR strains of *Acinetobacter baumannii*, *Pseudomonas aeruginosa and Enterobacteriaceae* have become particularly concerning due to reports from hospitals all over the world [31]. Many causes have resulted in the trigger and spread of MDR bacterial species, including: abuse or misuse of current antimicrobial drugs, which has aided in the emergence of bacterial adaptive resistance mechanisms; a shortage of responsible antimicrobial stewardship, such that the use of numerous broad-spectrum medicines has contributed to the perpetuation of the cycle of rising resistance; and a lack of adequate infection control procedures [32].

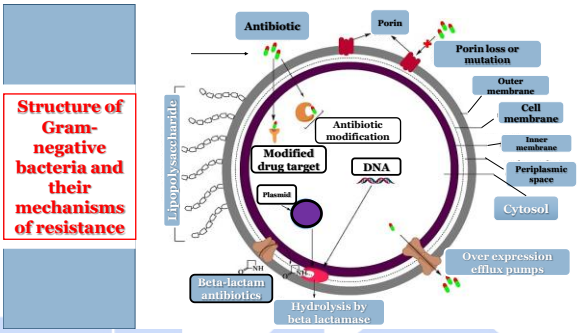

Fig. 5. Structure of Gram-negative bacteria and their mechanisms of resistance

3.1. Enterobacteriaceae

Some of the bacteria from the *Enterobacteriaceae* family are *Enterobacter, Escherichia coli, Citrobacter, Klebsiella, Serratia,Salmonella, Shigella, Proteus* [33]. These pathogens are a typical component of the gut flora and can be found in the human digestive system [34]. These bacteria can move into the circulation, posing a serious health risk like UTIs and diarrhoea [35]. Antibiotic resistance may develop in Enterobacteriaceae, it can be acquired as well as intrinsic [36]. Resistance in this bacterial species are common towards carbapenem group of medicines, which is last line of antibiotic antibiotic for the treatment of infections caused by MDR *Enterobacteriaceae* [37].There are two different types of bacteria that shows resistance to carbapenems namely Carbapenemase-Producing *Enterobacteriaceae* (CPE) and Carbapenem-Resistant *Enterobacteriaceae* (CRE) [10].

3.1.1. 3rd Generation Cephalosporin-Resistant-(Enterobacteriaceae)

Enterobacteriaceae produces lactamases that impart resistance to third-generation cephalosporins [13]. ESBLs, for example, can hydrolyze broad-spectrum cephalosporins, penicillins and monobactams etc [38]. Class Aβ-lactamases, including as TEM-1, TEM-2, and SHV-127, produce resistance to early-generation cephalosporins, amoxicillin, and ampicillin [39]. When genes encoding TEM-1, TEM-2,

or SHV-1 are altered, new β-lactamases are formed, leading in resistance to third-generation cephalosporins [40].
Cefotaxime hydrolyzing CTX-M and Carbapenem Hydrolyzing Oxacillinases (OXA), which are mainly seen in *P. aeruginosa* but only rarely in *Enterobacteriaceae*, are two more ESBLs that may be produced by *Enterobacteriaceae* [41]. Like ESBLs, AmpCβ-lactamases have found to have the capacity to degrade third-generation cephalosporin and are responsible to create resistant towards clavulanate and other β-lactamase inhibitors [40,41].

3.1.2. Carbapenem-Resistant – (Enterobacteriaceae)

CRE which is also known as Carbapenem-Resistant *Enterobacteriaceae* are a group of bacterial strains that possess resistance to carbapenem [42]. This is used for treating several severe infections [43]. This has been evidently found that apart from carbapenem, CRE (Fig. 6.) shows susceptibility towards many other antibiotics that are majorly used in regular common antibiotic intake and in some rare circumstances [44]. Antibiotic-resistant bacteria are sometimes also denoted as superbugs that potentially cause lung infections, skin and urinary tract infections [44]. This particular group of antibiotic-resistant CRE superbugs are capable of inducing infections and spread throughout the body with a rapid growth and even infect normal microflora or some beneficial microorganisms present in our body [45]. It causes severe illness by entering in human blood, bladder and in some other parts of the body where there are not supposed to be present at all [46]. It becomes difficult to treat. Apart from carbapenem, CRE *Enterobacteriaceae* isolates are also resistant to meropenem, ertapenem and imipenem etc [47]. Based on this, CRE can be classified into two sub categories such as carbapenemase-producing CRE (CP-CRE) and non-CP-CRE (non-CP-CRE) [17, 48].

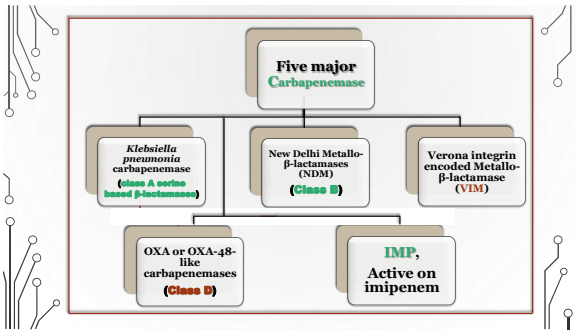

Fig. 6. A diagrammatic representation of five major Carbapenemases

3.2. Carbapenem-Resistant - Acinetobacter baumannii

Acinetobacter baumannii linked to nosocomial hospital acquired infections all over the world in humans are an aerobic Gram-negative in nature [16]. Resistance mechanism in these bacteria can be of many types.
β-lactamases enzyme produced by the bacteria inhibit β-lactams antibiotics, which is most common pathway present in *A. Baumannii* [48]. *A. baumannii*, can develop resistance by producing four types of β-lactamases which are A,B,C and D. These bacteria can acquire resistance genes and can integrate those gene in its own genome to produce these all four β-lactamases [49]. TEM-1, SCO-1, and CARB-4 are some gene which are responsible to produce narrow-spectrum β-lactamases, whereas genes name GES-11 and CTX-M, are responsible for the hydrolysing the ESBL leading to reduce carbapenem sensitivity [21] [20]. Penicillins, cephalosporins, and cephamycins are all susceptible to Class C β-lactamases [19]. In *A. baumannii*, AmpC cephalosporinase is present naturally [18]. Penicillins, cephalosporins, and cephamycins are all ineffective against Class C β-lactamases [19]. *A. baumannii* has AmpC cephalosporinase. Lengthened cephalosporins and carbapenems can be hydrolyzed by Class D or OXAsβ-lactamases, which prefer oxacillin [20].

- Efflux pump systems overexpression is one more method through which *A. baumannii* are resistant to a variety of treatments, notably tigecycline and imipenem [21]. The four kinds of efflux pumps are the RND superfamily, the MFS superfamily, the MATE family, and the Small Multidrug Resistance (SMR) genus transporters [8]. Tigecycline susceptibility is reduced by overexpression of the RND type AdeABC efflux pump [21].

- The enzymes that cause *A. baumannii's* resistance to aminoglycosides include acetyltransferases, acetyltransferases, and phosphotransferases [23]. Aminoglycosides are metabolised by these enzymes. Plasmids, transposons, and integrons can all carry coding genes [48].

- Changes in membrane permeability induce permeability faults [16]. Porins are peptides that form passage in the outer membrane to enable molecules to pass through, and they play a key role in the resistive mechanism [17]. In *A. baumannii* reduced expression of many porins, including such Caro and Omp are main cause of carbapenem resistance [11]. Sensitivity towards last resort drug Colistin in *A. baumannii* is due to changes in lipo polysaccharide (LPS) layer on which actually colistin works to kill the bacteria, reduction in membrane integrity happens [47].

- Antibiotic target sites are modified by target site changes including penicillin-binding proteins (PBPs), DNA gyrase mutations, and others [46]. As found in quinolone and tetracycline resistance in *A.*

baumannii, overexpression of particular PBPs promotes imipenem resistance as well as alterations in DNA gyrase [45].

- Integrons are located in bacterial chromosome, or plasmids and are categorised into four categories [44]. Integrons have the ability to assemble and express genes related to antibiotic resistance, which makes them a good biomarker for *A. Baumannii* epidemic strains [43].

3.3. Pseudomonas aeruginosa

Pseudomonas aeruginosa is a gram-negative bacterium responsible for nosocomial hospital acquired resistance in critically ill person with low immunity [42]. This pathogen was first isolated in 1882 from pus. It can be transmitted to patients in hospital through breathing tubes, dialysis unit and other apparatus on which it can form biofilm for prolonged period of time. Genetic changes to alter efflux pump expression and change in outer membrane can lead to resistance towards common antibiotics, which are used to treat infections caused by *P.aeruginosa*. These resistances can be innate or can be acquired [49].

It is also considered as the fourth most frequent nosocomial pathogen identified, the second most prevalent cause of ventilator-associated pneumonia, and the third most common gram-negative bacteria that cause severe infections [41].

There are certain antibiotics such as cephalosporin, carbapenem, penicllin which are β-lactam antibiotics, that prevents the cell wall peptidoglycans synthesis in bacteria [11 Cephalosporins such as ceftazidime and cefepime which are 3rd and 4th generations of antibiotics are the most effective β-lactams that can use to treat *P. Aeruginoss* infections [40]. Antibiotic resistance in these bacteria is due to production of β-lactamases that breaks out the amide linkage in theβ-lactamring [5]. This renders the antibiotic to become inactive or useless [6]. There four distinct types A,B,C and D of β-lactamases that have been found in *P. Aeruginosa* [32] [30]. Among them the class B in required to induce the functions of zinc cation functions [29, 32]. In that case, it also requires MBLs along with this class B β-lactamase [40]. The rest of the types help in inactivation of β-lactams through serine-residue catalysis [39].

Several β-lactams, including benzylpenicillin, imipenem etc are able to produce endogenous β-lactamase, including AmpC β-lactamase [47]. A gene mutation that causes overexpression of AmpCβ-lactamase in *P. aeruginosa* can result in resistance [10]. ampR, a positive transcriptional regulator required for the induction of β-lactamase [22]. On other hand, ampG, a transcription factor, acts as 1,6-anhydromurapeptides permease that efficiently induces ampC [23]. ampD is a cytosolic N-acetyl-anhydromuramyl-L-alanine amidase which is responsible for repressing ampC expression [47] [13]. AmpE is a cytosolic protein that functions as a sensory converter protein required for induction [44].

FQ resistance develops when a variation in the microbial chromosomes' gene producing DNA gyrase occurs, or when a medication is actively transported out from the cell [38]. Colistin was shown to be more successful than otherβ-lactam medicines in treating MDR *Pseudomonas* infections and it was even more effective when aztreonam, imipenem, ceftazidime, piperacillin or ciprofloxacin were combined with colistin [47]. Fosfomycin in combination with cephalosporins, and penicillins has exhibited much potential in the treatment [46].

3.4. Helicobacter pylori

Helicobacter pylori are present in human gut responsible for the cause of gastritis, peptic ulcers, and stomach cancer [18]. These bacterial species are also found to be exhibit MDR causing trouble to health practitioners to treat these diseases [19]. Clarithromycin is a drug which were first line treatment for the infection caused by *H. pylori* but now this species have developed resistance to this antibiotic, reason behind is changed in 23S rRNA gene, which decrease the drug's affinity [20]. Target site of *H. pylori* such as penicillin-binding proteins (PBPs) alterations, DNA gyrase mutations are also reason behind drug resistance in [21]. Imipenem resistance and DNA gyrase mutations are caused by overexpression of certain PBPs, as seen in *A. baumannii* quinolone and tetracycline resistance [23, 48].

Other factors responsible are efflux pump overexpression, transcriptional factor IF-2 mutation and the ribosomal protein L22 mutation [48]. According to WHO susceptibility towards Clarithromycin is high priority among antimicrobial research and innovation [17]. Integrons' exceptional ability to combine and expression of drug resistance genes makes them a good biomarker for detection of *A. Baumannii* epidemic strains [47].

3.5. Campylobacter- Fluoroquinolone-Resistant

Campylobacter is tiny, motile, and curved gram negative bacterial species [46]. These rod-shaped bacteria, which belong to the *Campylobacteraceae* family, are thermophilic (30°C to 46°C) and microaerophilic (5 percent O_2) [45]. *Campylobacter* is a zoonotic disease that lives in the intestines of domestic and wild animals and birds [46,48]. It can infect humans through drinking contaminated water, eating raw or uncooked meat, drinking unpasteurized milk, and coming into touch with infected animals or (rarely) humans [22]. Human infections are spread by reservoir

hosts like as poultry, cattle, sheep, pigs, birds, dogs, and cats [43]. In both developing and developed countries, this food-borne bacterial pathogen is the main cause of gastro-enteritis and septicaemia in humans [42].

Infections caused by *Campylobacter* can range in severity from a simple, self-limiting disease to a potentially fatal infection [49]. Treatment of self-limiting intestinal infections with fluids and electrolytes is adequate, but antibiotics should be used for severe extra-intestinal *Campylobacter* infections such septicaemia, endocarditis, and septic thrombophlebitis [41]. Antimicrobial resistance in *Campylobacter species* has risen in both developing and wealthy nations in recent years, and it is now a global concern [49]. It's worth noting that penicillin, cephalosporin, and sulphonamide resistance has arisen [11]. On the other hand, the world health organization (WHO) demonstrated in February 2017, that, fluoro-quinolone resistant *Campylobacter species* are on the rise throughout the world, indicating that finding effective medicines is a top priority [40].

3.6. Shigella spp. -Fluoroquinolone-Resistant

In developing as well as developed countries, *Shigella* is a group of human-adapted pathogenic bacteria that causes dysenteric diarrhoea (*Shigellosis*) [39]. The cure of shigellosis is advised using a class of antimicrobials known as fluoroquinolones, but tolerance to this class of antimicrobials is quickly increasing within this genus [38]. *Shigella* is the most common cause of diarrhoea worldwide, affecting young children disproportionately in impoverished nations and high-risk populations in wealthy countries [13]. *Shigella* infections are currently treated with antimicrobials, most often fluoroquinolones, to alleviate discomfort and prevent disease transmission [37]. Since the turn of the century, resistance to fluoroquinolones has arisen in many *Shigella species* (*S. dysenteriae, flexneri, and sonnei*), first in endemic places and then extending to non-endemic areas [10]. Despite the fact that they emerged at separate times, the evolution of fluoroquinolones in these *Shigella species* has significant similarities in terms of epidemiology and resistant mechanisms [36].

4. Treatment

Antibiotic resistance is a serious and growing problem [12]. More than 700,000 deaths each year, as per centres for disease control and prevention is due to MDR with the marking expected to climb to about 10 millions by 2055 [35]. Urgent need to develop new strategies and antibiotic agents is needed and considered a high priority [34]. Advancements in this direction includes focus on natural products, with natural product screening being reintroduced to find novel therapeutic agent to combat infections caused by MDR resistant bacteria [33]. In this section, we'll look at a few new revolutionary medicines (Fig. 7.) that have emerged as a result of research and development programmes for use against gram-negative bacteria that are resistant towards antibiotics [32].

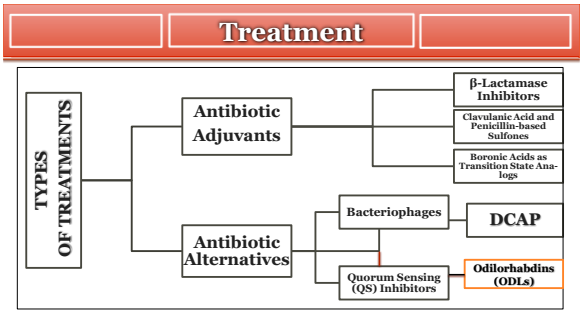

Fig. 7. Types of treatments in order to combat gram negative bacterial resistance

4.1. Antibiotic Adjuvants

These are known as 'resistance breakers' are chemicals with no effectiveness or a very minimal antibiotic activity by themselves [50].These adjuvants are when in combinations with other antibiotics in a medication therapy, it boosts up or enhances the efficiency of the antibiotic inducing a blockage or resistance for the treated bacteria to that particular drug [31]. Antibiotic adjuvants have helped in revitalizing the usage or application of several antibiotics against resistant organisms [29]. This in turn has reduces the need of novel, challenging and expensive antibiotics discovery as the efficiency can be resurrected by adding adjuvants only [24].

3 kinds of antibiotic adjuvants that have been investigated mainly includes membrane permeabalizers, Lactamase inhibitors and Efflux pump inhibitors [4]. Antibiotic adjuvants work through one of four mechanisms to help medicines overcome bacterial resistance: drug enzyme inhibition, drug efflux by efflux pump transporter, absorption reductions related to permiabalization of membrane changes, and alterations in drug targets [33].

4.1.1. beta-Lactamase Inhibitors

These type of inhibitor(s) are prominent and common among health practitioners to combat bacterial resistance towards beta-lactam antibiotics [1] [2]. beta-lactamase inhibitors have retained the much more effective antibiotic adjuvants for more than 70 years, despite their long-term use [3].

Resistant bacteria develop β–lactamases [1]. This enzyme helps in the hydrolysis process acylation-deacylation-based β-lactam which is the core ofβ-lactam antibiotics [2]. These can be separated into twodistinct groups such as (1) Serineβ-lactamases and (2) MBLs. For Serineβ-lactamases, the

lactamase's nucleophilic serine moiety bonds covalently to a hydrolysed beta-lactam, are categorised as ambler categories A,or C, or D, and suppressed with the influence of sulbactam, tazobactam and clavu-lanic acid (CVL) [3,4]. MBLs, which belong to the ambler class B and carry active surface area including one or two zinc ions that attack β-lactams via polarised water molecules [5]. MBL are classed as B1, or B2, or B3 based on zinc ions and sequence [6]. MBLs are usually significant against all beta-lactam antibiotics, with the exception of monobactams. For class B MBLs, there are presently no approved inhibitors [7].

4.1.2. CVL Acid and Penicillin-dependent Sulfones

Streptomyces clavuligerus naturally produces culvulanic acid which is a β-lactam [8]. In the year 1976 this was identified that *Streptomyces clavuligerus*is able to produce clavulanic acid and first time this was used to inhibit beta-lactamase in combination with amoxicillin [14]. CVL acid has structural resembles identical to penicillin as it connect to lactamase by covalent bond [2], [15]. No clinical impact against class B,or C, and D beta-lactamases is observed [25].

Zidebactam is studied to be a β-lactamase inhibitor which is bicyclo-acyl hydrazide [26]. A zebibactam/cefepime together, is now under phase 1 clinical trials [27]. In trials it is being checked whether it can treat any infections caused by Gram-negative MDR microorganisms [28].

4.1.3. Boronic Acids as Transition State Analogs

Serine proteases are inhibited by boronic acids. A number of azide analogues are potent inhibitors of class A and C20β-lactamases [9]. They primarily block the enzyme by forming a ternary complex with the serine residue, which are similar in formation of the β-lactamase-catalyzed hydrolytic process thus confusing the inhibition [29].

Many boronic acid compounds have been designed to targetβ-lactamases rather than other cysteine proteases [30]. The cyclic boronate ester RPX7009 (vaborbactam), which restored carbapenem activity against KPC-25, was the most efficient homologue [31]. Carbavance is a combination of a drug and biapenem and a compound RPX7009 is showing high potential and is in phase-three drug clinical tests for the treatment of MDR *Enterobacteriaceae* diseases[50].

4.2. Variety of Alternatives for Antibiotic

4.2.1. Bacteriophage(s)(BPs)

Bacteriophagesare considered as bactericidal viruses that only infect bacteria [32,49]. Felix d'Herelle named and discovered bacteriophages in 1917 [33]. The use of bacteriophages for treating several infections is a traditional, less effective, old concept eclipsed the during the antibiotic golden age [34,35,48]. In recent discoveries, with antibiotic resistance at an all-time high, BPs therapy is gaining traction [35]. With the advent of enhanced modern technologies and genome-sequencing, identification and generation of novel BPs therapeutics is gaining attention [12].

Bacteriophages are bactericidal agents that impair many, if not all, bacterial activities [36]. BPs possesses high or increased level of strain or species specificity, allowing them to avoid dys bacteriosis and secondary-infections [10]. BPs cannot infiltrate eu-karyotic cells, and does no harm to the cell no studies is recorded thus far [37]. In case of bacterial cell pahges amplify using bacterial DNA and also enhances the antibacterial actions [13]. Phage, unlike traditional antibiotics, may penetrate and remove biofilm [38].

New phages can be invented at a lot inexpensive or reasonable cost and in a much shorter time frame than antibiotics [39]. Conducting sufficiently designed, randomised, controlled clinical trials for phage treatment application is a challenge [40]. The majority of these trials could not able to attract participants or achieve meaningful results [11]. So far, there are very few publications on clinically usable bacteriophages have been published [41].

The most current bacteriophage clinical trial was a study to see if Phago-Burn (a combination of 12 natural lytic anti-P21) was effective and tolerable [49]. It was studied that burn wound infection(s) induced by *Pseudomonas aeruginosa* can be cured with the help of bacteriophages in an alginate template [46,46,49]. This has been found that phage cocktail concentration had minimised after production, and individuals received a comparatively lower dosage than expected [47]. This has resulted, the phage cocktail to minimise the bacterial burden in the burn sites more slowly than standard therapy [17]. The experiment was discontinued on January 2, 2017 due to lack of potency [16].

On the other hand, resistance against phages of bacteria is unavoidable [48]. Bacteria that are resistant to a specific phage can still be infected by others [23]. Despite the fact that phage are potent to aid in treating infections particularly those emergences of resistant bacteria, some research suggests that phages aid in evolvement and antibiotic - resistant strains, as phages could be used to accumulate, sustain, and spread bacterial infections genes [2].

4.2.2. DCAP (2(3,6-Dichloro-9H-carbazol-9-yl)-2-hydroxypropylamino)-2-(hydroxy-methyl)-propane1-3diol

In 2012, Hurley and colleagues was the first one to propose this molecule as an antibacterial agent [19]. This shows promising effect in killing bacteria different strains such as *E. coli* and *Pseudomonas sp.* [18]. DCAP was discovered via an elevated search for substance that suppresses the action of MipZ, an ATPase that potentially can control the devision sites in *Caulobacter crescentus* throughout vitro [19]. DCAP acts as membrane inhibitor that promotes

cellular injury for different bacterial species through two mechanisms: one is that it elevates transport of ion through the membrane, thus modulating ionic charge in membrane, and second, it lowers lipid bilayer permeability [20]. It is an antibiotic with the extra advantage of killing dormant bacteria & biofilms [1]. It differs from those other membrane-active drugs because of its sensitivity for bacterial membranes [2]. It has little effect on the membrane of red blood cells, and when tested for its effects on mammalian viable cells, it only showed a decrease in viability at high doses and after more than 6 hours [3] [4].

4.2.3. Quorum Sensing (QS) Inhibitors

The genes cluster in bacterial genome regulates processes to survive and cause infections like sensitivity towards antibiotics, formation of biofilms which can be named as microbial chemical communications (quorum sensing) (Fig. 8.) [5]. Autoinducer-2 (AI-2) studied to govern and induces intra- and interspecies bacterial communication [6]. QS Mediators examples are oligo-peptide in gram-positive bacteria as well as N-Acyl homoserine lactones (AHLs) in GNBB species. (S)-4,5-dihydroxy2,3-pentanedione, or (S)-DPD is QS modulation found in both type of bacterial sp. [7,21]. LsrK phosphorylates DPD, is a substance present in bacteria which activates the process [14]. Different types of DPD variations in their core structures can suppress LsrK thus can be used as antibacterial agents [24]. Isobutyl-DPD and phenyl-DPD in combination with gentamycin can act synergistically [24]. Virtual screening revealed 2-substituted aminobenzoic acids, also acts as inhibitors of the communication [15]. (Fig. 9.) [25]. QS modulation by different compounds has emerged to be the most potent treatment for a variety of bacterial virulence characteristics like biofilm, motility, EPS (exopolysaccharide production) etc. [26]. QS Inhibition in combination with other medicines can be used to address antimicrobial resistance [27].

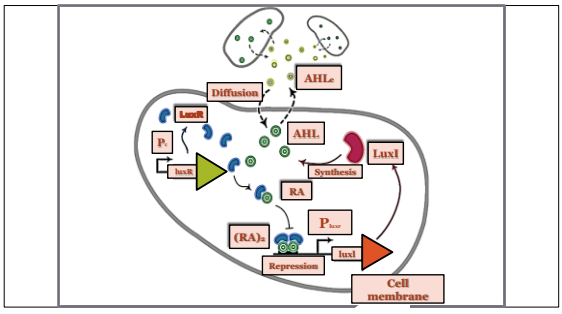

Fig. 8. A diagrammatic representation of quorum sensing in gram negative bacteria

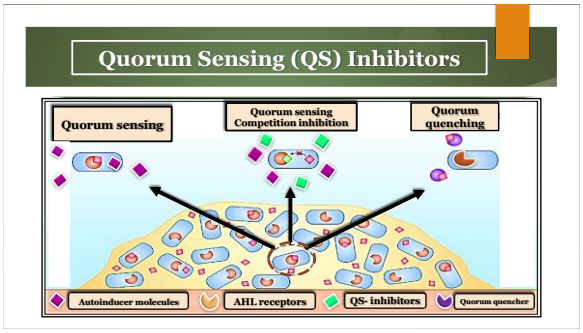

Fig. 9. Targeting quorum sensing. Schematic of QS in bacteria as well as methods to block this signalling mechanism

4.2.4. Odilorhabdins (ODLs)

ODLs are also able to kill both types of bacterial sp., especially ceftazidime-resistant *Enterobacteriaceae* [28]. By attaching to bacterial ribosomes and making contact with rRNA and tRNA, ODLs these type of treatment acts as inhibitors, by decoding the translation machineries and increases the affinity aminoacyl tRNA and ribosomes [9]. Studies have shown promising results have been obtained that suggests ODLscould be a good place to start when it comes to developing ODL clinical candidates [29].

5. Patient Education

Antibiotics possess no effectiveness treating cold and other viral infections or diseases [30]. Instead, they induce other bacterial growth as a side effect which becomes challenging to destroy [31]. Prolonged use of same antibiotics even develops antibacterial resistance where they become no longer effective against those bacterial strains [50]. Improper dosage, misuse of antibiotics, improper intake may also result in development of resistance [32].

Bacterial resistance is often described as antibiotic resistance [34]. Even the most powerful and higher dosage of antibiotics may possess no effectiveness against some microorganisms that develops resistance power when used for prolonged period [35]. This is an emerging problem worldwide [36]. It is described as "one of the world's most critical public health obstructions" by the centre of disease control and prevention (CDC) [31,36]. It is a particular concern in low-income and developing countries [10]. This is due to the fact that health-care providers in the region frequently lack swift, useful diagnostic methods for determining which illnesses are caused by bacteria and which are not [37].

To reduce the possibility of resistance emergence in bacteria and to avoid antibiotic overuse:

- Inquire with your doctor about whether your child's condition is bacterial or viral [13]. Discuss the potential harmful effects and benefits of antibiotics [38]. Inquire about treatment options if infection is caused due to

virus [40]. Pressure of prescribing the antibiotics on your doctor is not correct [39].

- Allow illnesses which are less severe (particularly those caused by viruses) to recover on its own in due time [11]. This helps to control the use of antibiotic and thus helps in spreading antibiotic-resistant [41].
- Antibiotics must be taken for the exact duration told by the doctor [42]. Otherwise, the infection may reappear [45].
- Don't let your youngster have antibiotics for any more days than the prescribed one [46].
- Do not utilise leftover antibiotics or keep extra drugs "for the next time" [48].
- Don't give youngster the same antibiotics that are for another family member or adult [23].

6. Future aspect

Antimicrobial resistance is undeniably increasing [18]. If left untreated or unchecked then it can become a serious threat to the pharmaceutical industry as well as in the entire medical sector [19]. As mentioned earlier that misuse of antibiotics, frequent or prolonged use of same antibiotic, improper dosage or miss dosage of the drug can result in the bacterial resistance development [20]. This also induces the need of discovery of new antibiotics but the same can repeat when resistance will develop [21]. Thus, a thorough research and development through a series of trial-and-error method, development of combination therapy with adjuvants or phages are at emerging needs [22]. As seen above, there are a plethora of intriguing, possibly beneficial techniques to building or discovering novel anti-bacterial, but only a small number of these have resulted in the approval of therapeutically viable drugs [48]. Rather than being a shortage issue or crisis, it can be even referred as the crisis of invention [46]. According to pharmaceutical industry insiders, the biggest hurdles for effective clinical development of novel antibiotics are facing high degree of challenges as it is a real expensive process and have lot of regulatory issues to come across [17]. The low return of the investing amount is the foremost challenge in the continuation of research and development and invention of new antibiotics [47]. On addition, this requires high level of regulations such as novel trial phase designing in national and international level, new inventions to the novel regulations in the global platform, engagement of private as well as public partnerships for maintaining the public health concerns related ethics [46]. When it comes to the development of MDR bacteria treating antibiotics, the process becomes complex and expensive which is hindering the overall process of invention of new antibiotics [45]. Due to this, it takes around 10-15 years for an antibiotic to become marketed after its actual discovery [45]. During this span, new development of strains, new resistance capacity development also interrupts and hinders the initiatives [22]. Thus only 2% antibiotics in the preclinical development reach up to the phase 1 trial and the same can hardly reach to the subsequent authorisation process for human use [43]. During this phase trial methods, a lot of compounds get rejected or subjected to discontinuation because of their high toxicity level, undesirable pharmacokinetic profiles, low activity, development of further bacterial resistance, commercial or strategic consideration issues etc [38].

7. Conclusion

Gram-negative antibiotic-resistant bacteria are a potential threat to entire medical field related to antibiotic assay and infection control study and to the entire pharmaceutical industry as the development of antibacterial resistance to an antibiotic by a bacterium or by a group of bacterial species or strain can be a real threat. This has induced the emergence of synthesizing new antibiotics which is challenging, expensive and time-consuming process. Novel techniques to overcome gram-negative bacteria's innate and acquired resistance provide promise for the future. Therapies such as, use of combination therapy of beta–lactamase inhibitors and antibiotics have exhibited good results in growth inhibition of resistant strains of gram-negative bacteria. This has shown both bactericidal as well as bacteriostatic effect against different bacterial strains.

Incorporation or use of bacteriophages as the natural selection process has also shows promising effect to this new approach of antibiotic development against several resistant strains of gram-negative bacteria. This approach has shown successful result in destabilizing several distinct bacterial processes as well as the ODLs.

One more promising trend is to look to nature for antibacterial agents that target targeted therapies, such as bacteriophages, that are either bactericidal virus that destabilise a variety of bacterial processes, as well as ODLs, novel hybrid peptide antibiotics. This usually gets attached to small monomers of bacterial ribosome. In addition to this, the misuse of antibiotics without actually knowing the root cause of the disease, or improper dosage, or improper timing of intake of antibiotics is also potential cause that can induce resistance. People must be aware and should be educated about how to use antibiotics. The time gap should be maintained strictly when used for a span of time in an antibiotic course. A proper rules and regulation the distribution process should be followed to avoid the chances of resistance development.

The misuse of antibiotics causes antibiotic resistance to spread throughout the world. It is necessary to educate people, so efforts must be made. The Gram-negative MDR microorganisms are the most dangerous, thus all of these new medications and procedures are necessary to tackle resistance

to antibiotics and MDR variants. In order to control the increase in MDR Gram-negative infections, the development of novel antimicrobials and combination of new and older medicines should be invented for the priority disease listed by WHO. Governmental support and effort is needed to make novel antibacterial drugs successful throughout the world. Governmental support is much needed to stimulate research and development. Resistance pattern in local area is needed for the comprehensive antimicrobial stewardship. In terms of the efficiency of futuristic antimicrobial therapy, strict protocols must be followed for wrong prescription of antibiotics and abuse of antimicrobial drugs.

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