Review Article

MECHANOSENSITIVE CHANNELS: UTILISING THE BACTARIAL CELL'S DEFENCE MECHANISM AGAINST IT

ABSTRACT: Mechanosensitive are integral membrane proteins in bacterial cell membranes as well as archaea, and eukaryotes. The role of these bacterial mechanosensitive channels is essential for protecting cells from structural damage during hypoosmotic shock. Mechanosensitive channel of small conductance (MscS) and mechanosensitive channels of large conductance (MscL)are the predominant channels in *E. coli*. Activation of mechanosensitive channels typically occurs when the bacterial membrane senses tension or distortion. Overall, the ecological niche of bacteria shapes the selective pressures acting on mechanosensitive channels, leading to their adaptation to specific environmental conditions. Bacterial mechanosensitive channels contribute to the ability of bacterial pathogens to adapt to and survive within the host environment, as well as to modulate interactions with host cells and tissues during infection. They could also act as entrance gates for specific antibiotic classes into bacterial cells. Accordingly, it was discovered that nitrofuran nifuroxazide and tuberactinomycin viomycin depended on both Ec-MscS and Ec-MscL for enhanced efficacy. Several compounds have been identified that directly target mechanosensitive channels. For example, ramizol has been shown to reduce the gating threshold of MscL and MscS channels, while styrylbenzene inhibits MscL channels in *S. aureus*, *Streptococcus pneumoniae*, and *Clostridium difficile*.Bacterial mechanosensitive channels are versatile drug targets due to their role in promoting bacterial virulence and host colonization, serving as entry points for antibiotics, and being structurally distinct from mammalian counterparts.These special structures which are meant to protect the cell can also be harnessed as drug targets, thereby increasing the susceptibility of the bacterial cell to target antibiotics. This feature has been harnessed to reduce the burden of antibiotics resistance and its attendant effect on global healthcare complications.The aim of this review is to examine the various physiological, drug resistance and pathogenicity roles of these mechanosensitive channels as well as their usefulness as potential drug targets.

KEY WORDS: Mechanosensitive, Channels, Osmolality, Gating, Protection, Antibiotics, Targets, Susceptibility

Introduction: Mechanosensitive channels could be regarded as integral membrane proteins in bacterial cell membranes as well as archaea, and eukaryotes [1]. They are embedded in the membrane bilayer of prokaryotic cells as shown in figure 1[2]. They are essential for protecting cells from inherent structural damage during hypoosmotic shock. These devices are dormant under normal physiologic and environmental conditions and become activated in times of a

perceived or impending mechanical-membrane stretch [3]. The role of these channels in bacterial cells is basically that of an emergency-release valve during alterations in the existing osmolarity, as, e.g., an experience when there is a sudden influx of water into soil bacteria after heavy rainfall, these channels prevent the cell form bursting by releasing osmolytes to counter the increased turgor pressure [4]. These channels are distributed uniformly over the cytoplasmic membrane(Fig. 1) and protect cells by a non-specific release of solutes, thereby balancing the osmotic potential for the entry of water[4]. These channels are activated by mechanical stimuli, such as changes in membrane tension, and play vital roles in maintaining cellular homeostasis during osmotic stress [1]. It could be summarized that bacterial mechanosensitive channels are essential for protecting cells from structural damage during hypoosmotic shock [4].

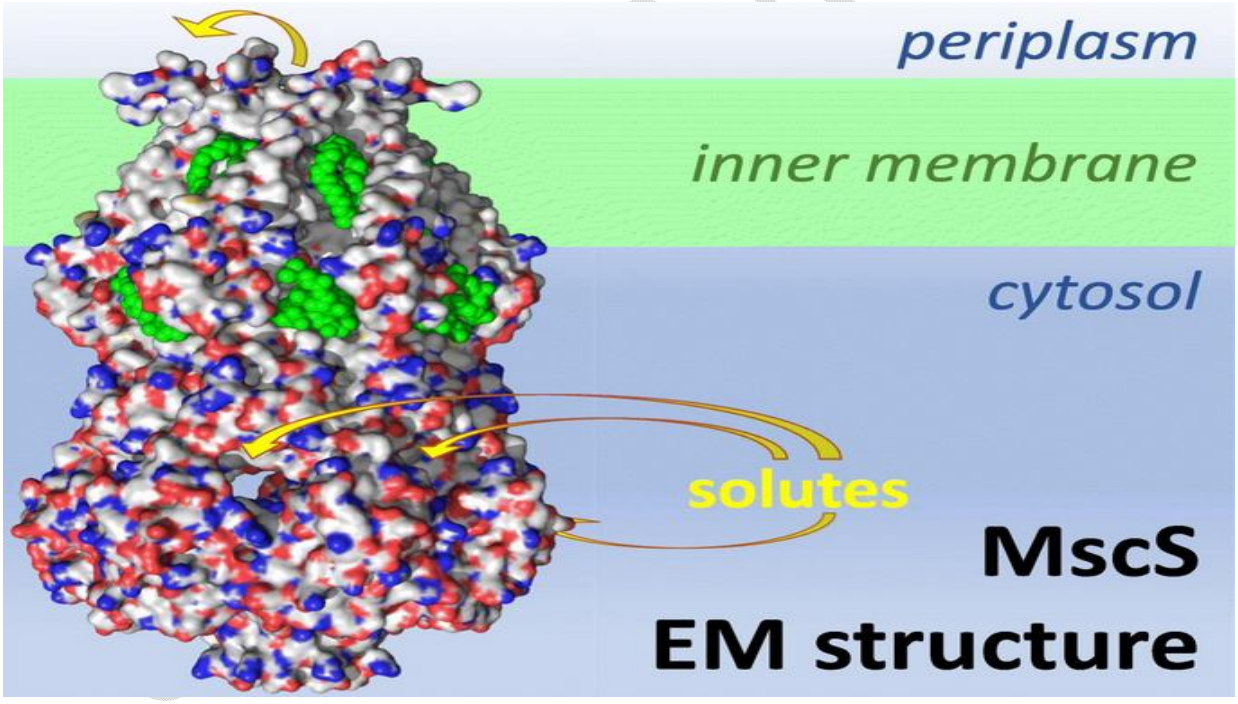

Fig. 1: Structure of the Mechanosensitive Channel MscS.
Adapted from Rasmussen*et al.,*[2].

Types of mechanosensitive channels

Bacteria, like *Escherichia coli* is capable of maintaining a positive turgor pressure in the range of 2 and 4 atm [5]. The organism can resist changes when the turgor temporarily goes above 10–20 atm. **Mechanosensitive channel of small conductance (MscS) and mechanosensitive channels of large conductance (MscL)** are the predominant channels in *E. coli*[6], which are considered the most implicated in osmoprotection. MscS channels are non-specific cation channels with a small conductance [7], while MscL channels are also non-specific cation channels but with a larger conductance. MscS channels can be further divided into three subgroups: MscS, MscK, and YbdG. MscS channels are the most abundant and widely distributed in prokaryotes [8]. MscK channels are found in some bacteria and are activated by both mechanical stimuli and voltage while YbdG channels are the least studied and are found in only a few numbers of bacteria. In summary, bacterial mechanosensitive channels can be classified based on their structural, molecular weights and functional properties. Here's a classification based on these aspects:

1. **Large Conductance Mechanosensitive Channels (MscL)**:
    - MscL channels are characterized by their large conductance, allowing the passage of ions and small molecules in response to mechanical stimuli[9].
    - They typically have a wide pore diameter, enabling the release of osmolytes to relieve flutuations in osmotic pressure[10].
    - MscL channels are homologous across bacterial species and are often encoded by the mscL gene[11].
    - The gating mechanism of MscL has been extensively studied through structural and computational models[12], which suggest that the channel undergoes a conformational change in triggered by changes in membrane tension, leading to the expansion of the pore and thereby releasing solutes and ions [8].
    - **Molecularweight** of approximately 136 kDa in its pentameric form (each subunit is about 27 kDa)[13]
2. **Small Conductance Mechanosensitive Channels (MscS)**:
    - MscS channels have a smaller conductance compared to MscL channels.
    - They play a role in responding to less severe changes in membrane tension and are involved in fine-tuning bacterial responses to mechanical stress[14].
    - MscS channels are diverse across bacterial species, with multiple paralogs present in some organisms [15].
    - MscS is a homoheptameric protein with three transmembrane domains per subunit
    - **Molecular Weight** of Approximately 31 kDa for the monomeric form, with the functional heptameric form being around 217 kDa[16].
3. **Mechanosensitive Channels of Small Conductance (MscK)**:
    - These channels have distinct structures from the MscL and MscS.
    - They have been identified in some bacterial species and help in osmoregulation and in responding to impending mechanical stress [2].
4. **MscCG Channels**:
    - These channels are found in *Corynebacteriumglutamicum* and are structurally similar to MscL channels[17].
    - They are involved in osmoregulation and response to mechanical stress in this species. [18]

5. **Bacterial Mechanosensitive Ion Channels of Large Conductance (MscCG)**:
    - MscCG channels are present in various bacterial species and are characterized by their large conductance.
    - They play a role in osmoregulation and response to mechanical stress, similar to MscL channels [19].

Activation of mechanosensitive channels

As stated earlier, mechanosensitive channels in bacteria play a crucial role in responding to mechanical signals like changes in osmotic pressure or membrane stress [20]. These channels allow ions, such as potassium and sodium, to cross the bacterial membrane in during mechanical perturbations, helping to regulate cellular osmolarity and prevent cell rupture.

Activation of mechanosensitive channels typically occurs when the bacterial membrane senses tension or distortion. The precursors of this tension varies and may include changes in osmotic pressure, physical deformation of the membrane, or interactions with external forces [21].

The exact mechanisms of mechanosensitive channel activation vary depending on the specific channel type and the bacterial species [22]. However, many mechanosensitive channels are known to be gated by alterations in membrane tension. When the membrane is stretched, it activates conformational changes in the channel protein, leading to its opening thereby allowing ion flux across the membrane. The MscL channel found in *Escherichia coli* and other bacteria is activated by perceived membrane stretch and functions like a safety valve to avoid cell death under conditions of high osmotic pressure or mechanical stress [8]. The mechanism of action of prokaryotic mechanosensitive channels involves an iris-like increase in pore dimensions during channel opening triggered by membrane tension [23].

As reported by [23], the membrane of the bacterial cell functions as a flexible sensor, converting an applied force input into an output signal that is processed by mechanosensitive channels within the cell. The information can be utilized by the cell for several purposes including maintenance of cellular viability when osmotic stress is present and controlling turgor pressure.Both gram-positive and gram-negative bacteria have been shown to exhibit the hypoosmotic-induced release of osmolytes [24].

The importance of understanding the activation of mechanosensitive channels in bacteria is not limited to elucidating basic cellular physiology but also has implications for related fields, like microbiology, biophysics, and biotechnology [23]. The ability to manipulate these channels could potentially be used to develop novel strategies for controlling bacterial growth or engineering bacteria for desired applications [21].

Physiological functions of Mscs and Mscl channels

Although it is evident that MscS and MscL channels permits the passage of solutes across the cell membrane as a response to membrane tension, research on the complete spectrum of physiological uses for the channel activity appears to still be in their early phases [21]. The section will address the potential applications of MscS and MscL homologs in bacterial cells to detect physiologically significant variations in membrane tension, translate the tension into transmembrane solute flow, and ultimately translate these transmembrane fluxes into actionable information.

According to [25], the main function of MscS and MscL type channels is to shield bacterial cells against osmotic shock as seen in figure 2. However, there are a lot of potential for functional specialization because many MscS-like proteins have been found in the genomes of bacterial and multicellular eukaryotic species [26].

Due to their acute osmotic sensitivity, both gram-positive and gram-negative bacteria must modify their cytosolic osmolarity in response to hypo- or hyperosmotic stress in order to prevent lysis or plasmolysis [21]. The bacterium *Corynebacteriumglutamicum* actively import and/or metabolize potassium glutamate and a variety of osmoregulatory organic solutes, such as proline, glycine betaine, and others, as the environment's osmolarity rises [18]. When bacterial cells experience an osmotic downshock, they must release these osmolytes to prevent lysis, which poses a risk. This buildup of solutes aids in maintaining cellular turgor under hyperosmotic conditions [1].

Gd^{3+} ions have been demonstrated to block MscL activity in *E. coli* spheroplasts, preventing lactose and ATP from leaving shocked cells. Subsequent research revealed that the shock-induced release of several cytosolic proteins needed the mscL gene[23]. The identification of yggB as the gene encoding MscS, however, was a crucial discovery and a link to cell viability; bacteria devoid of both MscS and MscL activities are nearly incapable of withstanding an osmotic downshock of 0.5 M[27]. The fact that YbdG, a standard gene when expressed supports mechanosensitive channel of miniconductanceMscM activity in *E. coli*which makes it necessary for the survival of less severe osmotic shocks adds more complexity to the situation[28]

According to [8], there is another recognized hypothesis that suggests bacterial membranes have several MS channels, each with unique tension thresholds and conductances, that work together to generate a gradient of responses. This explanation states that the first line of defense is made up of the smaller conductance, more tension-sensitive channels (such asMscS and YbdG), which open early in response to osmotic stress and "buffer" MscL from opening until it is absolutely essential. Additionally, it has been shown that homologs of MscL and MscS are necessary for *Bacillus subtilis* to survive osmotic shock [26], suggesting that gram-positive bacteria share similar role.

The benefit of this approach would be to preserve as much of the membrane gradients and cellular metabolites as feasible, while only opening a pore big enough to prevent the cell from rupturing [29]. But the degree of osmotic shock protection that a given channel offers does not seem to be directly correlated with its gating tension [5]. For instance, over-expressing wild type

YbdG in the same medium protects mscL- mscS- mscK-mutant cells from a 0.25 M osmotic downshock, but not from a 0.15 M shock [8]. These findings imply that the total number of channels present as well as the channel's structure may affect the critical threshold to open a channel.

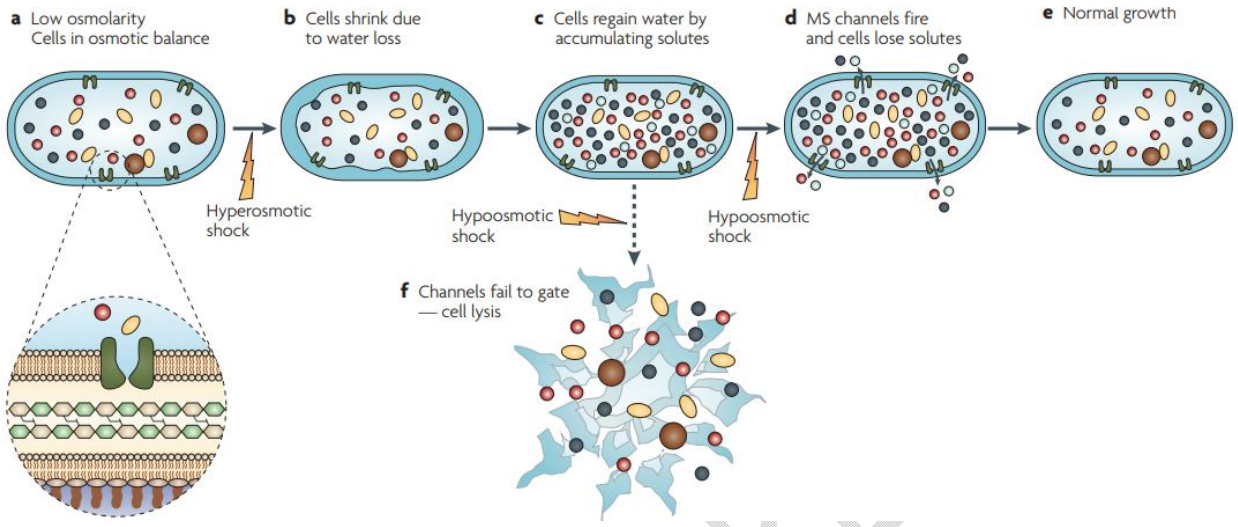

Fig. 2: Activation of Mechanosensitive channels by osmotic tension.
Source:[2].

Figure 2. Physiological function of mechanosensitive channels in bacteria. Physiological function of mechanosensitive channels in bacteria. In a Gram negative bacterium: a. Growth at low osmolarity accumulates sufficient K+ (red circles) and glutamate (black circles) to achieve an outwardly directed turgor pressure of ~4 atm. b | Cells lose water and shrink upon hyperosmotic shock. c | But recover full size by the accumulation of more K+ and glutamate with other compatible solutes (light blue circles). This makes the MS channels stay closed. d,e. At hypoosmotic shock, panel d) leads to the rapid entry of water accompanied by the immediate activation of the MS channels, which results in the loss of low molecular mass solutes (K+ , glutamate and compatible solutes) but the retention of large proteins and solutes (brown circles and yellow ovals) (panel e). f | If the channels are absent or fail to open, the influx of water generates high turgor pressure, which leads to the cell lysis. Adapted from [29].

Diversity of channels and niche-specific adaptability.

In this section, *E. coli* will serve as the model organism. According to [5], there are two main kinds of bacterial mechanosensitive channels, which were first identified by E. coli electrophysiological and then by the differences in their architectures. Six MscS homologues were found in *E. coli* by subsequent analysis, and each of these channels shows mechanosensitive channel activity. However, most of these channels are not frequently seen in electrophysiological tests and only have small but important functions in cell protection [8].

Different creatures have evolved distinctive solutions over time that represent their environmental niche [30].

Marine environment: MscL has been lost by several microbes that live their entire "normal" lives in marine habitats. According to [8], the deletion of the YbdG channel has the potential of increasing the rate dependency of cell death in controlled hypoosmotic shock and decreases the level of the salt concentration at which death occurs following a rapid downshock. Genome sequences demonstrated that bacterial genera differed significantly in their degree of complexity. Thus, many organisms have numerous MscS homologues, but the MscL channel activity is typically attributed to a single, relatively conserved gene. A cell that possesses many differentially expressed channels may be able to respond to hypoosmotic shock in a graduated manner.

Transgenic expression of *E. coli*MscL can protect certain marine organisms from extreme hypoosmotic stress [31]. Research has revealed that there is variety in the Vibrio species, and that only *Campylobacter jejuni* lacks MscL out of all the members of this species [32]. A requirement for protection during morphological changes may be indicated by the differential expression of homologues throughout bacteroid development.

The production and operation of mechanosensitive channels (MS channels) can be strongly influenced by the ecological niche occupied by bacteria. [32] describe the following ways that the ecological niche may affect mechanosensitive channels in bacteria:

1. **Osmotic Stress**: Bacteria residing in environments with fluctuating osmolarity, such as soil bacteria or those found in aquatic habitats, experience osmotic stress. Mechanosensitive channels help these bacteria regulate their cell volume by either removing or taking up osmolytes and ions in response to changes in osmotic pressure. The expression and activity of mechanosensitive channels may be upregulated in such environments to cope with osmotic challenges [29].
2. **Physical Confinement**: Bacteria living in physically constrained environments, such as biofilms or host tissues, experience mechanical stress due to confinement. Mechanosensitive channels are involved in sensing and responding to these mechanical cues, allowing bacteria to adapt their physiology accordingly. In such niches, mechanosensitive channels may be modulated to facilitate bacterial survival and persistence [21].
3. **Nutrient Availability**: Ecological niches vary in nutrient availability, and nutrient scarcity can induce stress in bacteria. Mechanosensitive channels may play a role in nutrient uptake by allowing bacteria to detect and respond to alterations in external nutrient concentrations. In nutrient-poor environments, the expression of mechanosensitive channels may be altered to optimize nutrient acquisition and utilization [19].
4. **Interactions with Hosts or Other Microbes**: Bacteria that interact with hosts or other microbial species may encounter mechanical stimuli generated by these interactions. For example, during infection, bacteria may experience mechanical stress from host immune responses [33]. Mechanosensitive channels can help bacteria sense and respond to these cues, influencing their survival and virulence. In polymicrobial communities,

mechanosensitive channels may also mediate interactions between different species by allowing the exchange of signaling molecules or metabolites in response to mechanical cues [34].

Overall, the ecological niche of bacteria shapes the selective pressures acting on mechanosensitive channels, leading to their adaptation to specific environmental conditions. Understanding these adaptations is crucial for elucidating bacterial physiology and ecology in diverse habitats.

Role of mechanosensitive channels during Infection

Bacterial pathogens interact with host cells and tissues during infection, sometimes coming into contact with the mechanical forces imposed by the host cells or tissues. Mechanosensitive channels could be involved in detecting and reacting to these mechanical stimuli, which could impact the host's bacterial adherence, invasion, and spread [35]. Overall, bacterial mechanosensitive channels contribute to the ability of bacterial pathogens to adapt to and survive within the host environment, as well as to modulate interactions with host cells and tissues during infection. A full understanding of the operations of these channels in the pathogenesis of bacteria will be helpful in facilitating the creation of innovative treatment approaches aimed at combating bacterial illnesses [36].

- First, they could encourage bacterial pathogenicity and colonization of hosts, function as portals for specific antibiotic classes to enter bacterial cells, or participate in a defense mechanism against antibiotic-induced stress [24].
- Second, they might be crucial for pathogenesis or virulence, or they might be a prime target for potential antibiotic molecules, which could boost antibiotic uptake or suppress bacterial defense mechanisms [24].
- Thirdly, through increasing antimicrobial-peptide action, they might potentially operate as host immunity potentiators[37].
- Fourth, the structural differences and conservation of mechanosensitive channels from their mammalian counterparts imply the possibility of developing selective inhibitors[37].
- Fifth, a number of substances have already been identified that either block or alter the activity of mechanosensitive channels, indicating that these channels are pharmacologically reversible [36].

Although the structural factors governing mechanosensitive-channel gating are constantly being discovered, their function as emergency-release devices that shield bacteria from hypoosmotic shock is well-established [29]. Because of this, they are frequently considered in relation to environmental issues, such as how soil bacteria like *B. subtilis* have adapted to periods of high rainfall [26].

But an increasing amount of data has emerged in recent years demonstrating their significance to harmful bacteria during infection [19]. Mechanosensitive channels, for instance, help cells adjust

to osmotic changes that happen when they move from the environment to the host and back. Additionally, they might be crucial in helping the body adjust to shifting osmotic circumstances. For instance, in bladder infections, a patient's water consumption can have a significant impact on osmolarity. Mechanosensitive bacterial channels are essential for the colonization, survival, and pathogenicity of bacteria, among other activities that are involved in bacterial infection. This will be summed up as follows:

Transition between Host and Environment: Pathogens must have appropriate adaption mechanisms to withstand the abrupt osmotic shift that occurs when they move from the environment to the host (and vice versa). The environment inside a human or animal body is typically more osmolar than that of many natural reservoirs, including freshwater [19]. For instance, in order for *Francisellatularensis*, the pathogen for tularemia in mammals, to survive the shift from its mammalian host to freshwater, it requires an MscS-like channel (Ft-MscS). In a similar vein, MscS homologs are required for *Campylobacter jejunii*, a primary cause of bacterial gastroenteritis in humans, to withstand the hypoosmotic stress which biulds during the pathogen's transfer from the host's digestive tract to the surrounding environment [38].

Mechanosensitive channels contribute to the persistence and spread of these diseases in the environment in both situations because they are required to preserve natural bacterial reservoirs. According to [38], *F. tularensis* is known to produce outbreaks of waterborne tularemia in a number of countries, and *Campylobacter* spp. are on the WHO's list of bacteria resistant to antibiotics, meaning that novel antibiotic therapies are desperately needed to combat these bacteria.Thus, it is critical to comprehend how mechanosensitive channels function during the transfer of bacteria from host to environment and how important they are for preserving natural reservoirs that permit the spread of these bacteria [23].

Mechanosensitive channels occasionally aid in the transfer of these bacteria from the environment to the host and allow them to settle in host tissues. *Salmonella typhimurium* serves as one illustration of this. Research has indicated that the mechanosensitive channel YnaI is necessary for colonizing the host's intestinal tract, and that *S. typhimurium* internalization increases when the ynaI gene is deleted [19].

Neisseria gonorrhoeae is another noteworthy example; it causes the disease gonorrhea by colonizing the mucosal membrane of the human urogenital tract. *N. gonorrhoeae* during infection may experience varying osmotic conditions, such as during the discharge of urine. It has been demonstrated that an MscS-like channel (Ng-MscS) is necessary for survival during osmotic downshock in this organism. In a murine vaginal-tract infection model, the *N. gonorrhoeae* wild type was seen to outcompete a mutant strain that lacked this channel in terms of colonization and survival, indicating the vitality of Ng-MscS in host colonization [39].

The human urinary tract as an osmotically demanding medium

Within the host, bacterial cells experience a variety of osmotic conditions, such as changes in osmotic pressure in various tissues or during immune cell phagocytosis [36];[3]. Mechanosensitive channels allow ions to enter and exit cells in reaction to changes in the osmolarity of the surrounding environment, aiding bacteria in maintaining osmotic equilibrium.

Bacterial survival in host settings depends on its capacity to control internal osmotic pressure [19].

Different osmotic circumstances are experienced by bacterial cells within the host, including variations in osmotic pressure in different tissues or during immune cell phagocytosis [40];[4]. Mechanosensitive channels help bacteria maintain osmotic balance by allowing ions to enter and exit cells in response to changes in the osmolarity of the surrounding environment. The ability of bacteria to regulate their internal osmotic pressure is essential for their survival in host environments [39].

The most well-known example of a human environment with sharply varying osmolarity is the urinary tract [40]. Urine is typically a complex, hypertonic environment with high concentrations of salt and urea, low pH, and both [19]. Urine in healthy humans usually contains the following: manganese, creatine (0.38–55.6 mM), sucrose (70–200 μM), amino acids, glucose (0.2–0.6 mM), citrate (1.0–2.0 mM), with traces of fatty acids. However, the osmolarity of urine can vary greatly based on a number of factors, including food, water intake, frequency of urination, and other medical disorders [41]. For instance, compared to bladder urine, kidney urine often has a lower pH and a higher osmolarity.According to [40], this fluctuation presents significant osmotic problems to bacteria that colonize the bladder and urethra. Uropathogens are able to live and even flourish in the urogenital tract, despite it being a harsh and difficult environment. These infectious agents rely on their osmoadaptive mechanisms to withstand the harsh osmotic conditions in this environment, and they use the contents of urine as nutrition [42].

Osmoadaptation mechanisms for uropathogens have been shown to be important in a number of investigations; however, most of these studies have focused on high-osmolarity-adaptation techniques [41]. For instance, [43] have demonstrated that the *E. coli* strain K-12 exhibits lower activity of the osmoregulatoryproline-transporter ProP than the *E. coli* pyelonephritis isolate HU734. *E. coli* HU734 did not grow as well in vitro in human urine when proP was deleted, but it did grow on a high-osmolarity minimum medium. Additionally, the removal of ProP decreased HU734 survival in the bladder[19]. Additionally, it has been demonstrated that OmpR, a component of the EnvZ-OmpR regulatory system that reacts to hyperosmotic stress, is necessary for the survival of uropathogenic*E. Coli* in the urinary tract [43]. It has also been suggested that urine osmolarity influences the virulence of uropathogens; *Pseudomonas aeruginosa* produced more virulence factors when urine osmolarity was raised from 200 to 300 mOsmol/L.

But with a further rise in osmolarity, a notable reduction in virulence factor production and growth was noted. Furthermore, compared to the same strain cultivated in nutritional broth, *P. aeruginosa* grown on a high osmolarity medium (300 mOsmol/L) demonstrated greater resistance to phagocytosis and increased virulence in a mouse model [42]. These findings demonstrate the critical role that osmoadaptive systems play in uropathogen development and colonization inside the urinary tract. Very little is known about the significance of low-osmolarity adaptation strategies like mechanosensitive channels, despite the obvious significance of hyperosmotic stress-adaptation techniques in urine [41].

However, given that urine's osmolarity can drop off quickly and significantly, for example, when consuming a lot of water, it makes sense to believe that mechanosensitive channels could also be involved in this situation. Future research on this topic will be intriguing, as it is supported by the apparent significance of Ng-MscS in *N. gonorrhoeae* colonization of the urogenital tract [19].

Effect on Antibiotic Susceptibility: Mechanosensitive channels have shown to be effectivein eliciting antibiotic in addition to their potential roles in virulence and pathogenesis [19]. Therefore, research has indicated that they could act as entrance gates for specific antibiotic classes into bacterial cells [44]. On the other hand, some antimicrobial compounds seem to be able to initiate mechanosensitive-channel opening, and it has been proposed that they are involved in stress adaptation and resistance to such compounds [45]. For instance, this was the case with the aminoglycoside dihydrostreptomycin, which binds to Ec-MscL and alters its conformation to permit both the influx of the antibiotic into the cell and the efflux of potassium and glutamate into the surrounding medium[46];[47]. Another aminoglycoside that showed a similar reliance on Ec-MscL was spectinomycin. These results imply that aminoglycosides share the ability to use MscL as an entry site, rather than being a characteristic exclusive to streptomycin. [45].

Antimicrobials that are affected by mechanosensitive-channel activity.

Research has indicated that the ability of aminoglycosides to eliminate bacterial persisters is enhanced when hypoionic shock is applied. Surprisingly, mechanosensitive-channel activators as parabens or indole significantly amplified this impact[48]. Moreover, it has been demonstrated that fast freezing increases the bactericidal action of aminoglycosides against persisters and other harmful bacteria [45]. This was explained by the instability of the cell membrane, which activated MscL and increased the uptake of aminoglycosides into the cells. It was proposed that this behavior is unique to aminoglycosides because it was not seen with β-lactams or fluoroquinolones [49].

Nevertheless, mechanosensitive channels have also been demonstrated to allow the entry of other substances into bacterial cells. Accordingly, it was discovered that nitrofuran nifuroxazide and tuberactinomycin viomycin depended on both Ec-MscS and Ec-MscL[50]:[51]. Similarly, it has been shown that curcumin, an antimicrobial component present in tumeric, exhibits action that is dependent on MscL but not MscS. Both in vivo physiology/flux investigations and patch-clamp study of native bacterial membranes have demonstrated that curcumin can activate Ec-MscL, suggesting that MscL also acts as an entrance channel for curcumin [52].

Furthermore, it has been proposed by recent research on MscL-specific agonists that tetracycline also demonstrates MscL-dependent action and may potentially enter this channel [44]. It will take more research to determine the true scope of this mechanism. Another lantibiotic with an unclear mechanism of action, sublancin 168, has similarly been shown to exhibit MscL-dependent antibacterial activity. Sublancin 168 susceptibility was shown to be osmolarity-dependent, with high NaCl concentrations decreasing the sensitivity of both B. subtilis and S. aureus. Although antimicrobials, particularly antimicrobial peptides, frequently display a salt-

dependent activity, the scientists were able to demonstrate that NaCl had no effect on sublancin 168's synthesis, activity, or stability [26].

Moreover, the lantibiotic was rendered resistant to a sensitive strain that had its mscL genes deleted, even in the presence of NaCl. It is unclear, though, if MscL functions as a target, an entry point for sublancin 168, or participates in some other mechanism that enhances the lantibiotic's bactericidal action [44].

Mechanosensitive channels in the context of the stress response to antibiotics.

Membrane-active antimicrobials including MP196, nisin, gramicidins, and aureins cause osmotic-membrane stability and decrease antibiotic susceptibility. Osmoprotective amino acids are released as a result of these agents' imitation of membrane stretch[19].

Other research has revealed that mechanosensitive channels may also function as a component of an antibiotic-stress response, which shields bacterial cells from antibiotics that target membranes, as opposed to situations where they increased the action of antibiotics [49]. Within the host, bacterial pathogens face a variety of stressful situations, including oxidative stress, exposure to antimicrobial peptides, and an acidic pH. By reacting to these shocks and adjusting the influx or efflux of ions, mechanosensitive channels help bacteria survive by preserving cellular homeostasis and lessening the harmful effects of stressors [19].

This was initially found for the antibacterial peptide MP196, which causes peripheral-membrane proteins to be displaced via altering membrane architecture [53]. Furthermore, this peptide caused *B. subtilis* cells to release glutamate and aspartate into the culture medium—a reaction that was likewise linked to osmotic downshock. Notably, pore creation was not the cause of this release, which was particular to these amino acids, and it was possible to mitigate its effects by removing the organism's four mechanosensitive channels [26]. Additionally, the mutant with the triple mechanosensitive channel was more responsive to MP196. Notably, [26] found that adding exogenous glutamate to the culture medium significantly reduced *B. subtilis*'s susceptibility to MP196.

Comparable results were observed with KCl and NaCl, indicating that osmostabilization was the cause of this action. Other membrane-targeting antimicrobial peptides, such as gramicidin A, gramicidin S, nisin, and aureins, were also found to cause a similar glutamate/aspartate release [19]. Similar to these findings, when exposed to penicillin, the industrial amino acid manufacturer *C. glutamicum* excretes glutamate via its mechanosensitive channels MscCG (NCgl1221) and MscCG2. Glutamate excretion is significantly reduced when both mscCG and mscCG2 are deleted; however, this effect can be recovered by complementing either of them[39].

Glutamate and aspartate were also reportedly exported by MscCG by passive diffusion, according to a patch-clamp study of a *B. subtilis* strain that was heterologously producing MscCG[19]. MscCG was found to prefer glutamate over aspartate in both investigations. It's significant to remember that heterologous manufacture of Ec-MscS similarly recovers ampicillin-induced glutamate excretion in the *C. glutamicum*mscCG deletion strain. Only when

certain treatments, including low biotin, the addition of fatty-acid ester surfactants (tween 40 and tween 60), or antibiotics that prevent the formation of cell walls, damage the cell membrane can *C. glutamicum* overproduce glutamate [54].

It was also noted that cells treated with antimicrobial peptides released more glutamate than aspartate, indicating that a similar preference may also exist for the *B. subtilis* channels, even though matching investigations on the *B. subtilis* proteins are scarce. It's interesting to note that the generation of glutamate in *C. glutamicum* triggered by penicillin resulted in the overexpression of genes producing MscCG and cellular defense systems. According to[55], a penicillin-stress response may involve transcriptional activation of several genes. However, it is still unknown how they relate to the synthesis and excretion of glutamate. Similarly, it has been demonstrated that ampicillin causes *E. coli* to produce mscL and mscS transcription. Other antibiotics including gentamicin, ofloxacin, and norfloxacin did not cause this induction, which is consistent with conditions that compromise the cell envelope[19].

According to [56], overexpression of MscS shields cells from sub-inhibitory ampicillin doses. It's interesting to note that changing its cytoplasmic domain reduces this effect. L-ornithine, L-arginine, and D-glutamate supplementation decrease ampicillin action, according to biochemical screens that measure the impact of various metabolite supplementations on antibiotic susceptibility. This is comparable to *B. subtilis*'s lower susceptibility to MP196 following glutamate supplementation [25]. It was hypothesised in a number of these experiments that the specific antibiotics and stressors promote mechanosensitive-channel opening by simulating a membrane stretch akin to that brought on by elevated turgor pressure. This theory would explain why the observed effects were limited to situations that compromised the cell membrane [25].

Biofilm formation: Many bacterial pathogens form biofilms, which are structured communities of bacteria encased in a self-produced mesh-like strucrure of extracellular polymeric substances (EPS). Mechanosensitive channels are involved in the regulation of biofilm formation by mediating the entry of ions necessary for biofilm development or responding to mechanical forces exerted on the biofilm structure.These biofilms are integral to bacterial survival, providing protection against environmental stresses, including mechanical forces[57].In the context of biofilm formation, mechanosensitive channels contribute to several key aspects:

- Sensing Mechanical Signals: Mechanosensitive channels detect changes in mechanical forces within the biofilm microenvironment. For example, they can sense shear forces generated by fluid flow or physical interactions between bacteria and their surroundings[19].

- Initiation of Biofilm Formation: Mechanical cues sensed by mechanosensitive channels can trigger the initiation of biofilm formation. Bacteria may respond to these signals by initiating the expression of genes required to produce EPS, adhesion to surfaces, and biofilm matrix assembly[58].
- Biofilm Maturation and Architecture: Once biofilm formation is initiated, mechanosensitive channels continue to play a role in the maturation and architecture of

the biofilm. They regulate processes such as cell-cell communication (quorum sensing), EPS production, and the development of complex biofilm structures[57].

- Adaptation to Mechanical Stress: Bacterial biofilms are subjected to various mechanical stresses, such as fluid flow in natural environments or shear forces in medical devices. Mechanosensitive channels help bacteria adapt to these stresses by modulating ion fluxes, cytoskeletal rearrangements, and gene expression patterns[57].
- Dispersal of Biofilms: In addition to promoting biofilm formation, mechanosensitive channels may also be involved in the dispersal of mature biofilms. Certain mechanical cues can trigger the activation of dispersal mechanisms, allowing bacteria to detach from the biofilm and colonize new environments[57].

Overall, mechanosensitive channels are integral components of the regulatory network governing bacterial biofilm formation. Understanding their role in biofilm development and maintenance could provide insights into novel strategies for controlling biofilm-associated infections and improving industrial processes[58].

Osmolarity and Host-Defense Peptides: This finding provides fresh insight into the salt sensitivity of antimicrobial peptides by showing that exogenous glutamate and salt supplementation, in addition to glutamate/aspartate release, appears to shield bacterial cells against membrane-active antibiotics. It is commonly known that high salinity conditions dramatically reduce the activities of various antimicrobial peptides[59]. This can be explained by either cations shielding negatively charged binding sites on the cell membrane or salt's electrostatic interactions with the peptides themselves [60].

Research have shown that salt may actually help to stabilize the cell membrane osmotically, which has the protective effect of preventing the action of antibiotics that act on the membrane and cell walls. Although the full implications of this possibility have not yet been determined, it is intriguing to talk about how this effect might impact host-defense peptides, for example, in an infection [60]. There are not many observations that could be linked to this kind of osmostabilization impact, especially when it comes to urogenital infections. For instance, urine with a greater osmolarity and lower pH greatly impairs neutrophils' ability to kill and phagocytose *Staphylococcus saprophyticus* and *E. coli*. When a pathogen enters the phagosome, host-defense peptides found in neutrophils are released [19];[32].

Furthermore, it is tempting to hypothesize that mechanosensitive channels not only aid in transitioning between media that possess different osmolarity but also provide protection against host-defense mechanisms like antimicrobial peptides, since multiple studies have demonstrated that these channels are necessary for host colonization or virulence [42].

Virulence factor secretion: Some bacterial pathogens secrete virulence factors, such as toxins or enzymes, to facilitate colonization and infection. Mechanosensitive channels may be involved in regulating the secretion of these virulence factors by modulating the membrane potential and ion gradients necessary for the proper function of secretion systems[61].

Mechanosensitive Channels as Novel Antimicrobial-Drug Targets:

It was noted not long after the discovery of antibiotics that bacteria might change and develop resistance to them. Antibiotic resistance in bacteria has increased because to widespread overprescription and use in agriculture, despite early warnings about the dangers of overusing them. These bacteria are commonly referred to as super-bugs since they are resistant to all current treatments [62]. As a result, efforts to combat the current antibiotic resistance threat are concentrated on developing new pharmaceutical entities and improving the stewardship of our available antibiotics. Antibiotics that exhibit a reduced propensity to induce resistance in bacteria are particularly noteworthy[63]. Antibiotics with multiple modes of action or combination therapies that need the defeat of two distinct processes appear to be one potential option [14].

Several compounds have been identified that directly target mechanosensitive channels. For example, ramizol has been shown to reduce the gating threshold of MscL and MscS channels in *S. aureus*, *Streptococcus pneumoniae*, and *Clostridium difficile*. Additionally, an MscL agonist moiety has been engineered onto antimicrobials or added as a separate compound to enhance antimicrobial activity and avoid drug resistance[19].

In summary, bacterial mechanosensitive channels are versatile drug targets due to their role in promoting bacterial virulence and host colonization, serving as entry points for antibiotics, and being structurally distinct from mammalian counterparts. Several compounds have been identified that directly target these channels, demonstrating their potential pharmaceutical effects [64]. Mechanosensitive channels are thought to play a significant role during infection, which means they may be used as a novel drug target. These functions include promoting bacterial virulence and tissue colonization, acting as entry points for particular antibiotic classes into bacterial cells, and being a part of a protective antibiotic-stress response [39].

Mechanosensitive channels may be important for virulence or pathogenesis or they may be a prime target for antibiotic potentiators, either by promoting antibiotic uptake or by inhibiting bacterial defense systems. This is true even though mechanosensitive channels are normally not essential in bacteria and their inhibition may not even cause growth defects in lab conditions [19]. By increasing the action of antimicrobial peptides, they could potentially operate as potentiators for host immunity. Moreover, due to unrestricted intracellular content leakage, channel gating modification into an "always open" state is really quite likely to be fatal [37].

Mechanosensitive channels are therefore very flexible pharmacological targets. Because mechanosensitive channels are physically different from their counterparts in mammals and are preserved, it may be possible to create inhibitors that are specifically targeted. Significantly, it is already known that a number of substances either suppress or alter the activity of mechanosensitive channels [23]. They do show that these channels are druggable, even though none of them have yet to be studied in a clinical setting. Although mechanosensitive-channel inhibitors have not received much attention in the development of antimicrobials or antibiotic potentiators thus far, they do represent an intriguing novel therapeutic target that should be investigated more in the future [65].

Compounds Directly Targeting Mechanosensitive Channels:

It has been discovered that the more potent derivative, IRS-16, and SCH-79797, bind to the MscL channel directly and cause membrane permeabilization when they activate it[45]. According to these results, an antibiotic compound's toxicity may be reduced and antibiotic resistance may be avoided by including or acquiring an MscL-activating component [7]. Not only was SCH-79797, a recently reported novel antibiotic molecule, effective against strains of bacteria resistant to other antibiotics, but it also showed to be extremely difficult for bacteria to become resistant to it[66]. This molecule has been shown to have two distinct mechanisms of action: one involves inhibiting the activity of dihydrofolate reductase, which is related to the metabolism of folic acid; the other involves enhanced membrane permeability and depolarization as seen in figure 3[67].

Additionally, in a mouse model of vaginal infection, a derivative called Irresistin-16 (IRS-16) showed enhanced potency, decreased toxicity, and demonstrated efficacy against *Neisseria gonorrhoeae*. Although one of its modes of action, membrane permeabilization, was well-described, the precise mechanism or mechanisms remained unknown [68]. There are other substances that have been created that interact with MscL and hinder its activity. Among the substances that target prokaryotic mechanosensitive channels are 011A, styrylbenzene, and ramizol. Mechanosensitive-channel activity has been demonstrated to be inhibited or modulated by these chemicals, indicating that these channels are druggable and have the potential to be novel targets for antimicrobial drugs [50].

MscL is a desirable target for therapeutic development due to its great conservation among bacteria and structural difference from mammalian mechanosensitive channels[69]. Since MscL does not have a selectivity filter, constitutive gating of this channel will be harmful to the cell since its wide pore will permeabilize the cell membrane in an unselective manner, which will cause the membrane potential to dissipate and ultimately lead to cell death [70].

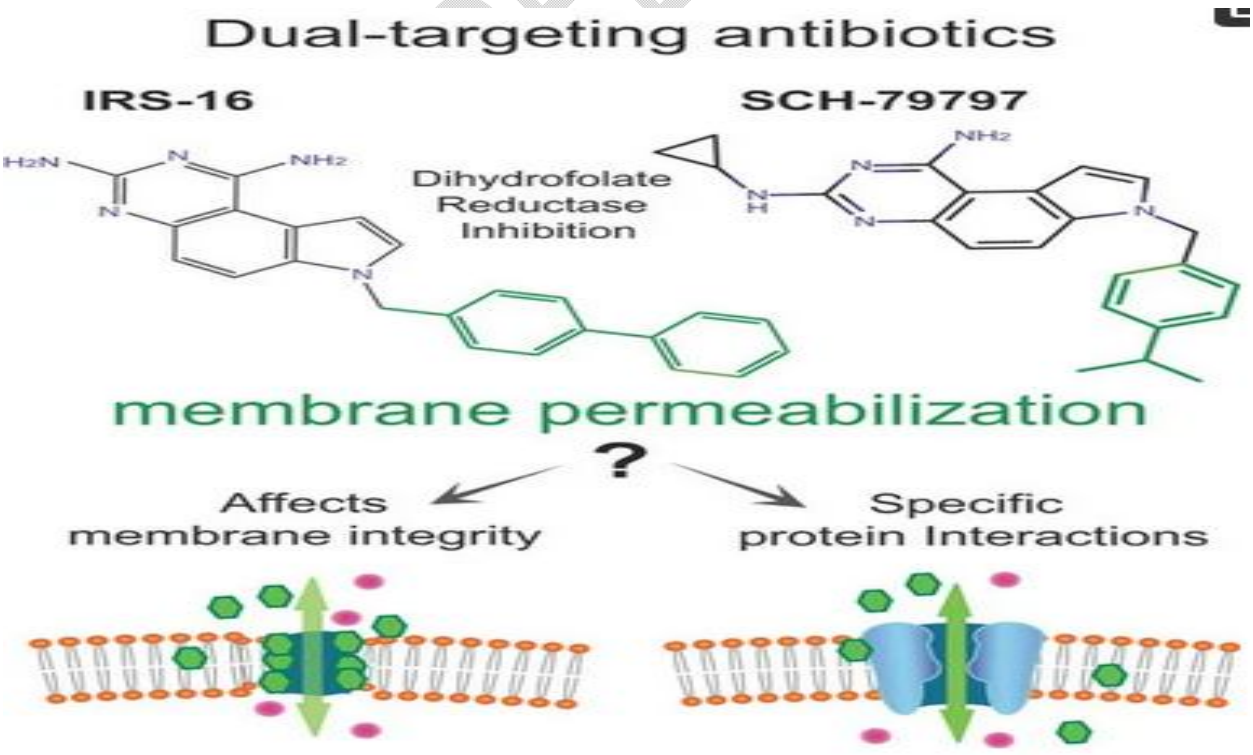

Fig. 3. This figure illustrates the dual targeting mechanism of the antibacterial compound SCH-79797 and its derivative IRS-16. one targeting the folate metabolism (black) by inhibiting the dihydrofolate reductase, and the other increasing membrane permeabilization (green). Demonstrating whether the effect is non-specific (bottom left) or dependent on the expression of bacteria-specific channels (bottom right) [14].

One of the first examples of a successful design of an MscL-targeting antibiotic compound is ramizol (formerly known as "compound 10"). Ramizol was identified through an in-silico screening method and was predicted to interact with MscL. In fact, the compound was demonstrated to inhibit the growth of *S. aureus* cells expressing MscL in vivo [4]. Moreover, ramizol also inhibited the growth of cells expressing MscS, albeit less successfully, indicating a nonspecific activation of mechanosensitive-channel gating in vivo. Furthermore, it is likely that ramizol's targets are not just MscL-sensitive channels; cells not expressing MscL or MscS were also inhibited when treated with higher concentrations of the compound, indicating an additional, concentration-dependent mechanism of action [45].

Patch-clamp tests, however, showed that ramizol only considerably lowered MscL's gating threshold, suggesting that it at least prefers this particular channel. Ramizol has been demonstrated to be effective in a *Caenorhabditis elegans* model of methicillin-resistant *S. aureus* infection, despite the fact that its exact mechanism of action is still unknown. This suggests that ramizol has the potential to be developed as a new therapeutic against antibiotic-resistant bacterial infections [71].

Further pre-clinical research has been conducted on ramizol, and the results on its effectiveness in treating Clostridium difficile infections, as well as its pharmacokinetic profile, dose, and drug-delivery methods, are encouraging. A distinct series of investigations has assessed the possibility of two MscL-specific agonists, 011A and K05, as new antibiotics [72]. These substances attach themselves to MscL, making it more susceptible to membrane stress. The treatment of *E. coli* cells with these chemicals resulted in reduced vitality due to incorrect gating of MscL. MscL-deficient cells showed resistance to various substances. Other bacteria, including *S. aureus* and *M. smegmatis*, have shown comparable effects [50].

It has been shown that the critical binding site for both drugs is a lysine residue at position 97 of Ec-MscL. When Bs-MscL, a mutant strain of *E. coli* lacking a lysine residue at the corresponding location, was expressed heterologously in a mscL-null strain, the strain became insensitive to both substances [19]. By substituting lysine for the matching Bs-MscL residue, it became susceptible to both agonists. According to reports, A011 and K05 can be used as adjuvant antibiotics since they can make kanamycin, ampicillin, tetracycline, and dihydrostreptomycin more powerful against *S. aureus* and *M. smegmatis*[66]. According to research, these substances selectively permeabilize the membrane by altering MscL gating, which makes it easier for antibiotics to enter the cytoplasm.

Although this could account for the pharmaceutical industry's enhanced activity of cytoplasmic-targeting antibiotics (tetracycline, kanamycin, and dihydrostreptomycin), it could not account for ampicillin's increased potency[73], as its target is on the outside of the cell membrane [66]. It is possible that the observed increase in activity is the result of a distinct combined effect of the agonists with ampicillin, since ampicillin stimulates the development of mechanosensitive channels in *E. coli* and penicillin activates them in *C. glutamicum*[74]. Through in silico

docking investigations, a structurally unique family of MscL agonists has recently been identified. These agonists target a binding pocket at the cytoplasm-membrane interface that is comparable to that of A011 and K05. While the potential for clinical development of such compounds remains to be exploited, these findingsshow the specific druggability of MscL[75].

Compounds Indirectly Modulating Channel Gating:

A number of compounds are known to modify channel gating indirectly by interacting with the lipid bilayer, in addition to molecules that directly interact with mechanosensitive channels [50]. In gigantic *E. coli* spheroplasts, for instance, it has been demonstrated that amphipathic molecules can activate mechanosensitive channels; the efficiency of these channels varies with their hydrophobicity. This is the case, for instance, with parabens, which are amphipathic substances utilized in the food and cosmetics sectors as antimicrobials. MscL and MscS have been demonstrated to activate spontaneously [76].

It's interesting to note that depending on whether parabens were given to the periplasmic or cytoplasmic side of excised membrane patches, they had distinct effects on MscS's sensitivity [76]. Parabens enhanced MscS sensitivity when applied to the periplasmic side of the membrane patch; however, this was not the case when parabens were introduced to the cytoplasmic side. It has been suggested that the cytoplasmic domain of the MscS gate may be the cause of this impact. Parabens increase tension in the inner leaflet and initiate channel gating when they are injected externally because they insert into the outer leaflet [19]. However, when parabens are inserted internally, they will raise the lateral pressure surrounding the channel, causing a "squeezing" effect that impedes[46].

Parabens have an unknown antibacterial mechanism, however it is doubtful that their major target is mechanosensitive channels because bacteria without these channels can still be susceptible [77]. The piscidins (P1 and P3), which are histidine-enriched, alpha-helical antimicrobial peptides that have been demonstrated to lower the activating tension of MscS and MscL in *E. coli* spheroplasts, are another class of amphipathic molecules that influence mechanosensitive-channel gating. According to [77], piscidins have the ability to alter the protein–lipid border either directly or indirectly. For instance, they may cause membrane stretch or curvature or reroute forces operating on the lipid bilayer to the protein, which would reduce the activation threshold. Nevertheless, the sensitivity of an *E. coli* mutant missing mscL, mscS, and mscK to piscidins does not significantly differ from that of the normal type, suggesting that mechanosensitive channels are not their main targets [78].

Another compound that is able to modulate mechanosensitive-channel activity is the spider venom GsMTx4, a globular amphipathic peptide. GsMTx4 has been reported to possess antimicrobial activity and is significantly more active against Gram-positive bacteria[79]. Interestingly, when the peptide was applied at the periplasmic side of the membrane patch, patch-clamp experiments showed a biphasic response of *E. coli*MscS and MscK: low peptide concentrations (2–4 μM) decreased the sensitivity of the channels to pressure, but higher peptide concentrations (>12 μM) had the opposite effect. It was demonstrated in a different investigation that GsMTx4 applied to the cytoplasmic side accelerates MscL and MscS opening rates. According to [80], this was explained by the peptide binding to the lipid interface, which locally increased the membrane tension and stabilized the mechanosensitive channels' enlarged shape[81].

Unlike GsMTx4, which has a variety of actions, gadolinium chloride ($GdCl_3$) only functions as an inhibitor, preventing the opening of mechanosensitive channels. It has been reported that in patch-clamp investigations of the mechanosensitive channels of E. coli, B. subtilis, and Enterococcus faecalis, administration of 100 µM GdCl3 entirely eliminates channel gating [82]. Further in vitro investigation showed that GdCl3 could only prevent MscL gating when anionic phospholipids were present, presumably as a result of their mediating role in the Gd3+ ion's interaction with the cell membrane [82]. It is believed that the gadolinium ions' interaction will cause lipid-bilayer compaction, which will raise lateral pressure and "squeeze" the channels into closing. GdCl3 traces on the membrane can be removed to reactivate mechanosensitive channels.

At the absolute least, these compounds are useful for understanding the function of mechanosensitive channels and have already significantly contributed to their characterization. However, the potential of these molecules as antibacterial drugs remains to be determined, potentially hindered by issues related to specificity and selectivity [19]. Although not much work has been done to develop these compounds as antibacterial medications thus far, given the ongoing antibiotic-resistance dilemma, they might reappear as promising new drug options.

CONCLUSION

Mechanosensitive channels remain useful structures in the regulation of osmotic pressure in bacterial cells. Their differentiation into different types enable the cell to effectively control the osmolality of cells by the controlled activation of only specific channels. These channels have also become targets for certain antibiotics as their presence or absence have been seen to either help or reduce the potency of specific drugs.

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