The unresponsive use of antibiotics led to the appearance of multiple drug-resistant bacteria strains. Studying the mechanism by which bacteria can resist antibiotics, the so called quorum sensing and biofilm formation, enabled the researchers to find bioactive compounds, derived from eukaryotes and prokaryotes. The disrupt of this mechanism is called quorum sensing inhibitors or quorum quenchers. This article provides an overview on the current research done on such bioactive compounds, the possible use of them as antibiotic alternatives, what are the advantage and disadvantages, the source from which it has been extracted, and how it may succeed to overcome bacterial resistance. The recommendation of researchers is to use some of these natural antimicrobial compounds combined to lower doses of antibiotics for treatment, the fastest way to limit the adverse effects of the exploitation of antibiotics and to avoid bacterial resistance.

**Keywords:** Bacterial resistance, Antibiotic adjuvants, Antimicrobial enzymes, Antimicrobial peptides, Essential oils, Phytobiotic.
exopolysaccharides (a major component of the biofilms), and virulent factors production. This gives the bacteria a chance to achieve specific actions that occur only when living in a community not when has been sporadic so that quorum sensing controls production and development of biofilms, virulence factors, sporulation, bioluminescence, and conjugation [33].

Biofilm and virulence disruption agents are called QQ or QSIs. They are considered a feasible alternative to antibiotics [34].

Three approaches were investigated to inhibit microbial QS:

First, disruption of autoinducer synthesis; second, the inhibition of ligand/receptor interactions; and third, degradation of the autoinducer through enzymatic destruction [35].

ADDRESSING ANTIBIOTIC RESISTANCE

A combination of antimicrobials may give synergistic effect due to the diversity of mechanisms which is necessary to overcome recurrent bacterial communication and kill persisting cells [36]. These mult.drug cocktails were not confined to antibiotics but extended to combinations of antibiotics with natural compounds that have the ability of QQ and act as non-antibiotic adjuvants.

This combination usage promotes the antimicrobial effect and prevents the bacterial resistance because the disruption of biofilm makes bacteria more disposed to even low doses of antibiotics [37]. The synergism between antibiotics and QSIs showed good results in regulating the resistant strains of Staphylococcus pathogenesis, where the bacterial sensitivity to the commercial antibiotics was elevated by RNAIII-resistant strains of S. aureus [38]. Administration of lysozyme in combination with oxacillin or vancomycin enhanced the antimicrobial effect against MRSA [40].

Among the polysaccharide hydrolyzing enzymes, lysozymes, alginicylases, dispersin B and amylases were reported. Lysozyme immobilized in chitosan was effective in inhibiting food spoilage microorganisms [41].

Alpha-amylase hydrolyzes existing biofilms of S. aureus [42]. Combination of proteases and amylases was effective in removing a Pseudomonas fluorescens biofilm [43].

The peroxidases, such as lactoperoxidases and myeloperoxidases, use the H₂O₂ to oxidize halides (bromide, chloride, and iodide) and isocyanate producing more potent antimicrobial compounds active against invading pathogens [44].

Antimicrobial Enzymes Derived from Bacteria

Quorum quenching enzymes that have an ability to degrade QS signal acylated homoserine lactone (AHL) autoinducers are AHL-lactonases, AHL-acylase, decarboxylases, and deaminase [9]. These enzymes were derived from different kinds of bacteria such as Actinobacteria, Rhodococcus, Achromobacter, Streptomyces, Firmicutes, Bacillus, Oceanobacillus, Anaeroba, Cyanobacteria, Proteobacteria, Alphabaculum, Comamonas, Halomonas, Hyphomycetes, P. aeruginosa, K. pneumoniae, Rabstonia, and Stappia [45-52]. The mentioned organisms have either AHL-acylase or -lactonase enzymes while Rhodococcus erythropolis might be the only organism which has both enzymes [53,54]. Interestingly, Bacillus thuringiensis does not produce the QS signal but produce AHL-lactonase [55]. Microorganisms that produce bacteriolytic enzymes (e.g., streptomyctetes) usually express a complex of several cell wall degrading enzymes with different specificities.

Lipase enzymes are considered an innovative and environmentally friendly approach for biofilm control due to their lytic and dispersal activities. Most of lipase enzymes used in industries are of microbial origin. It catalyzes the hydrolysis of esters for long chain aliphatic acids; several microorganisms produce lipases such as eukaryas, fungi, actinomycetes, yeast, bacteria, and archaea. Bacterial lipases include Bacillus, Penicillium, Staphylococcus, Pseudomonas, and Aspergillus [56].

Antimicrobial Enzymes Derived from Bacteriophage

Bacteriophages are viruses that replicate inside infected bacteria and then infect other bacterial cells. Endolysins, such as glucosidase, endopeptidase, and lipase, act as non-antibiotic adjuvants.

Table 1: History of The Development of Antibiotic Resistance [16]

<table>
<thead>
<tr>
<th>Years</th>
<th>Developed</th>
<th>Showed bacterial resistance</th>
</tr>
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<tbody>
<tr>
<td>Before 1935</td>
<td>Sulfonamides</td>
<td>Sulfonamides</td>
</tr>
<tr>
<td>1941-1945</td>
<td>B-lactam (penicillin)-streptomycin</td>
<td>B-lactam (penicillin)</td>
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<tr>
<td>1946-1950</td>
<td>Chloramphenicol-tetracycline</td>
<td>Tetraacycline</td>
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<tr>
<td>1951-1955</td>
<td>Erythromycin</td>
<td>Streptomycin-chloramphenicol</td>
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<tr>
<td>1956-1960</td>
<td>Vancomycin</td>
<td>Vancomycin</td>
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<tr>
<td>1961-1965</td>
<td>Cephalosporins-quinolones</td>
<td>Cephalosporins and quinolones</td>
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<tr>
<td>1966-1970</td>
<td></td>
<td>Erythromycin-vancomycin</td>
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<td>1986-1990</td>
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<tr>
<td>1996-2000</td>
<td>Oxazolidinones (linezolid)</td>
<td>Oxazolidinones (linezolid)</td>
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<td>2001-2005</td>
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and transglycosylase, showed bacteriolytic activity against Listeria monocytogenes, Bacillus anthracis, Staphylococcus, and Clostridium butyricum [4]. Furthermore, they can clear some Gram-positive bacterial infections such as Enterococcus faecalis and Clostridium perfringens, [58]. The pairing of an antibiotic with a bacteriophage adjuvant is currently used and available in Georgia. Combination of ciprofloxacin and a lytic phage cocktail is currently produced, by PhagBioBioDerm, in a biodegradable polymer matrix. Amidase PAL and endopeptidase CP1 from phage CP1 are synergistically capable to control the systemic pneumococcal disease [59,60]. Endolysins separated from phage phi3626 can treat Clostridium contaminations [61]. The type PAL of endolysin can kill the Streptococcus Group A. The type LYS endolysins kill Staphylococcus, especially methicillin safe S. aureus [62]. Endolysins PyhV12 demonstrates a decent lytic movement against vancomycin, safe E. faecium, Enterococci, and E. faecalis [63].

**Antimicrobial Enzymes Derived from Animal**

Quorum quenching enzymes have been isolated from animals such as rats, mice, and zebrafish.

Porcine kidney acylase I inactivated QS signals and prevented the formation of biofilm in Pseudomonas putida and Aeromonas hydrophila [64]. Mammalian paraoxonases have hydrolytic effect on esters and lactones [65]. Mammalian type of lactonases differs from that derived from bacteria as the first type needs calcium ion to be active [65]. Epithelial cells of human have the ability to inactivate the autoinducer, AHLs, synthesized by P. aeruginosa [66].

Foods such as chicken breast, turkey patties, beef steak, beef patties, and homemade cheeses revealed inhibition for the Gram-positive bacteria autoinducer (Al-2) activity by 84.4–99.8% [67]. These QSIs vary in their effect on the expression of virulence-related genes [68].

Pancreatic lipase enzymes catalyze fatty acid synthesis in bacteria; therefore, it can serve as a potential antibacterial agent that is effective against many bacterial strains [69]. Lactonase, AHL acylase, and oxidoeductases are from mammalian paraoxonase. They are QSIs and can modulate P. aeruginosa infection [70].

**Antimicrobial Enzymes Derived from Plants**

Laccases, are QSIs enzymes, have been found in plant extracts derived from fruit, flowers, leaves, and bark of Laurus nobilis, Combretum albiforum, and Sonchus oleraceus [71,72]. Allinase and thiol enzyme group separated from garlic and other medicinal plants act as QSIs [73,74]. Lactonase presents in clover, lotus, legumes, peas, yam beans, and alfalfa showed AHL degrading abilities [75,76]. Papaya (Carica papaya L.) is rich in cysteine protease enzyme which has a crucial role in many vital antimicrobial processes in living organisms [77].

**Quorum Quenching Enzymes Derived from Marine Organisms**

Algae like Laminaria digitata has bromoperoxidase enzyme that has QQ activity by oxidation process to AHL signal group [30C,HSL] [78]. Delisea pulchra contain halogenated furanes which similar in shape to bacterial AHLs and can block the receptors (LuxR) and hinder QS process [79,80].

Alginate lyases are enzymes, found in algae, invertebrates, and marine microorganisms, used in combination with gentamicin to control P. aeruginosa in the respiratory tracts of patients with cystic fibrosis [81-83].

**Antimicrobial Digestive Enzymes**

Digestive enzymes supplemented to improve the feed efficiency ratio and stimulate the absorption of nutrients, also affect on the bacterial population in the alimentary tract [84]. Some of these enzymes such as carbohydrates and phytases were synthesized and are commercially sold as feed additives to monogastric animal [85]. These enzymes will affect on the nourishment of intestinal flora which will compete the other pathogenic or harmful types of bacteria [84]. Furthermore, when xylanase and lysozyme enzymes were added to broiler chicken diet, it minimized the gastrointestinal lesions of C. perfringens in the ileum [86,87].

In conclusion, combination of certain types of enzymes, polysaccharide-degrading enzymes, D/Nases, proteases, and anti-quorum sensing enzymes, is required for successful control of microbial infections. Unfortunately, industrial enzyme production is somewhat expensive, especially for biomedical applications where pure enzymes are required [88].

**ANTIMICROBIAL PEPTIDES (AMPs)**

AMPs are found among all living organisms as a component of the innate immune response [90,89]. Most of the reported AMPs were of animal origin such as glycine/arginine-rich peptides, tachyplesin, brevinin peptides, and alpha- and beta-defensins [91]. In plants, few AMPs have been isolated from seeds, roots, stems, flowers, and leaves from various species and have demonstrated activities against different pathogens such as viruses, fungi, bacteria, protozoa, and parasites. Thioins, defensins, lipid transfer proteins, puroindolines, and snakins were different groups of AMPs reported in plants [92] - >800 different AMPs with the same biological activity to the naturally occurring AMPs have been designed and engineered from natural nucleic acid sequences [93] or selected from online combinatorial libraries [94].

Bacterial resistance against AMPs is apparently more difficult to be emerged in comparison with existing antibiotics as they have several targets and several modes of actions [95]. However, some bacteria developed resistance against human AMPs during evolution [96,97]. Hence, plant AMPs could be better than human ones because they rarely contact human pathogens to induce such resistance.

AMPs range from 4 to about 40 amino acids in length, engineered AMPs are identical to natural ones and all of them are hydrophobic and cationic in nature. It plays its role inducing changes in membrane permeabilization, destabilization, inhibition of macromolecules synthesis, intracellular translocation of the peptide, and inhibition of DNA/RNA/protein synthesis [98]. As polycationic peptides, AMPs interact electrostatically with negatively charged bacterial surface structures including lipoteichoic acids, and then, they interact with the lipid bilayers of the cytoplasmic membrane forming transmembrane pores and resulting in weakening of the membrane [99]. AMPs exert its effect on microbial plasma membranes, within few seconds of addition. Then after, within 1 h, bacterial membrane vesiculation, fragmentation release of DNA, cell aggregation, and destruction of cell morphology were noted. Thus, AMPs should rapidly pass through outer membrane thick proteoglycan layer of Gram-positive bacteria and the lipopolysaccharide layer of Gram-negative bacteria [100].

It is apparent not always biomembrane permeabilization is required for AMPs activity as it can translocate inside microbial membranes as well. This translocation results in membrane leakage and may occur at low concentrations of AMP before inducing permeabilization. For example, both the defensin cryptdin-4 of human and the AMP magainin 2 offrog translocate across bacterial cell wall bilayers within average 10 min [101,102].

**AMPs Derived from Bacteria**

The bacterial enzymes peptide synthetases produce the AMPs such as polymyxin, gramicidin, bacteriocin, and sugar peptide. The polypeptide polymyxin is obtained from Bacillus polymyxa. It is effective against different pathogenic bacterial species such as P. aeruginosa, Salmonella, Escherichia coli, and K. pneumoniae. The polypeptide bacteriocin is effective against Gram-negative cocci and spirochetes. Bacteriocin has been used commercially as feed additive for animals combined with bacitracin methylene salicylic acid and zinc [103]. There are several products of bacteriocins such as nisin, fermentacin, subtilcin, plantacin, helvetacin, lactacin, and sakacin that have antimicrobial effect against resistant pathogenic strains [104]. Bacteriocin can kill bacterial cell by interfering its protein metabolism on molecular bases.
Many bacteriocins are applied as bacteriostatic in food products [105] as it can inhibit foodborne pathogens such as Clostridium botulinum, S. aureus, Bacillus spp., L. monocytogenes, and E. faecalis [106,107].

**AMPs derived from plants**

Major AMPs reported in plants were 5–13 kDa. Such small AMPs were shown to demonstrate good antibacterial activity against some bacteria such as S. aureus, E. coli, K. pneumoniae, and P. aeruginosa. Plant AMPs like other AMPs have higher activity against Gram-positive than Gram-negative bacteria [108-110]. Different kinds of antimicrobial substances were reported such as saponins [111], canavanine, and some important antifungal defenses [112]. Antimicrobial activity of AMPs obtained from wheat endosperm was reported against S. aureus and Micrococcus luteus [113]; other studies showed that extracts of AMPs from different germinating and ungerminated seeds of Psam sativum, Punica granatum, Cocos nucifera L., and Phaseolus vulgaris showed broad spectrum of antibacterial activity against Micrococcus luteus, S. aureus, Staphylococcus epidermidis, Salmonella typhi, K. pneumoniae, E. coli, Proteus vulgaris, Pasteurella multocida, and P. aeruginosa [114].

**AMPs derived from marine organism**

Marine green growth is rich in peptides and high assorted proteins [115]. Crypteins, the recently produced peptides, have novel therapeutic effects [116,117]. Brown seaweed Saccharina longicuris is rich in AMPs (>10 kDa MW) resulting from trypsin enzymatic hydrolysis and extracted using HPLC. Other subfractions of peptide precursors were identified such as ubiquitin, leucine, and histone that play a part of the innate immune defense of the seaweed [118].

**PLANT EXTRACT & ESSENTIAL OILS (EOs)**

Plant materials, known as phytobiotics, have been introduced in animal nutrition as antioxidative, antimicrobial, anti-inflammatory, and antiparasitic factors [119,120]. The phytobiotic compounds were classified into phenolics/polysaccharides, alkaloids, terpenoids/EOs, and lectins/polypeptides [121]. Plant extracts exert the antimicrobial effect at MIC 100–1000 μg/ml in vitro [122]. These phytobiotics have different modes of action against pathogens. First, tannins act by iron deprivation and enzymes interactions [123]. Second, cryptolepine may act as DNA intercalator and an inhibitor of topoisomerase enzyme [124]. Third, saponins act on the bacterial membrane by binding with sterols causing membrane damages and deformity of cells [125]. In the same time, some plant compounds act as QSI as their chemical structure is like those of AHL so it can bind its receptors (LuxR/LasR) [126]. Furthermore, degradation of AHL signal takes place under the effect of γ-aminobutyric acid which promotes the bacteriolytic enzyme, lactonase [127]. Flavonoids such as kaempferol, naringenin, quercetin, and apigenin work as QSIs by inhibition of the QS autoinducers, HA-1 or AI-2, mediated bioluminescence in Vibrio harveyi [128]. Catechins produced by herbal plants like tea can stimulate AHL-lactonase and clear the plasmid of E. coli [129]. Furocoumarins and rosmarinic acid present in grapefruit juice and the roots of sweet basil corrupt the biofilm formation by E. coli and P. aeruginosa, respectively [130,131]. Thymol is currently used in combination with vancomycin and EDTA as antimicrobial [132]. Furthermore, the combined effect of the antibiotic, tobramycin, and some plant extracts (cinnamaldehyde and baicalin hydrate as QSIs) was effective to clear the infected lungs with Burkholderia cenocepacia and P. aeruginosa [133-135]. The use of EO to combat epidemic infection by multiresistant bacteria is promising. The EO of lemon, thyme white, cinnamon, and lemongrass showed high antibacterial effect against some problematic strains such as Streptococcus, MRSA, and Candida strains [136,137]. The synergistic effect between the EO and antibiotics was reported where Mentha piperita, Thymus vulgaris, and Rosmarinus officinalis oils when combined with ciprofloxacin showed more efficient antimicrobial effect [138]. Hydrophobicity of EOS is the main reason of increased bacterial cell permeability and consequent leakage of cell constituents [139]. Followed by disturbance of vital functions such as nutrient processing, synthesis of structural macromolecules, secretion of many growth key enzymes and protein synthesis. EO disrupts and lowers the intracellular PH of bacteria and consequently impairs many crucial cellular processes such as DNA transcription, protein synthesis, and enzymatic activity [140]. Furthermore, the production of ATP in prokaryotes occurs both in the cell wall and in the cytosol by glycolysis. Hence, the alterations on intracellular and external ATP balance will be affected due to the action of the EO on the cell membrane [141].

The anti-QS activity of EOS or its components was reported as they affect the autoinducer expression [142].

**CONCLUSION & RECOMMENDATION**

We have to put in mind that alternatives to antibiotics should be non-toxic, easily excreted from the body and have low residues, not stimulate bacterial resistance, be stable and do not decompose inside GIT, do not cause environmental pollution, have good taste, and kill the pathogen without destroying the normal flora.

Actually, till present, there is no antibiotic alternative that meets all the above-mentioned criteria. All proteinaceous compounds, such as feed enzymes and AMPs that have been put into market as well as bacteriophage lysins, enzymatic biofilm inhibitors, and quorum quenching enzymes under development, are naturally unstable and easily degraded in the digestive tract. On the other hand, antibiotics can directly kill bacteria or inhibit with better antibacterial effect than all antibiotic replacements. Antibiotics are made by single and relatively pure active ingredient with consistency, high stability, and quality ensured by good manufacturing practice. However, researchers appreciate the combination of more than antimicrobial compound to avoid the development of bacterial resistance. Combination of biofilm inhibitors with antibiotics showed good results than when used sporadic. Hence, we have to put in mind that we have to use the natural antimicrobials to prevent than to cure disease or in combination to antibiotics as adjuvant to improve its function and waiting for updates from scientific research.

**AUTHORS’ CONTRIBUTIONS**

Emtenan Mohamed is specialized in veterinary medicine and her area of interest is to find natural product that may improve animal health and decrease the risk of disease. Enas is specialized in enzymology. Hence, both authors shared in throwing light on most antimicrobial products and the last updates in this respect. Editing and scientific revision were done by Emetenan.

**CONFLICTS OF INTEREST STATEMENT**

Authors declared that there is no competing interest between them.

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