



AKADÉMIAI KIADÓ

European Journal of
Microbiology and
Immunology

11 (2021) 1, 10-17

DOI:

10.1556/1886.2021.00003

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Antibacterial properties of capsaicin and its derivatives and their potential to fight antibiotic resistance – A literature survey

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Received: February 16, 2021 • Accepted: March 9, 2021

Published online: March 24, 2021

REVIEW PAPER



ABSTRACT

Antibiotic resistance is endangering public health globally and gives reason for constant fear of virtually intractable bacterial infections. Given a limitation of novel antibiotic classes brought to market in perspective, it is indispensable to explore novel, antibiotics-independent ways to fight bacterial infections. In consequence, the antibacterial properties of natural compounds have gained increasing attention in pharmacological sciences. We here performed a literature survey regarding the antibacterial effects of capsaicin and its derivatives constituting natural compounds of chili peppers. The studies included revealed that the compounds under investigation exerted i.) both direct and indirect antibacterial properties *in vitro* depending on the applied concentrations and the bacterial strains under investigation; ii.) synergistic antibacterial effects in combination with defined antibiotics; iii.) resistance-modification via inhibition of bacterial efflux pumps; iv.) attenuation of bacterial virulence factor expression; and v.) dampening of pathogen-induced immunopathological responses. In conclusion, capsaicin and its derivatives comprise promising antimicrobial molecules which could complement or replace antibiotic treatment strategies to fight bacterial infections. However, a solid basis for subsequent clinical trials requires future investigations to explore the underlying molecular mechanisms and in particular pharmaceutical evaluations in animal infection models.

KEYWORDS

capsaicin, capsaicinoids, capsinoids, antibacterial effects, synergistic antibiotic properties, multi-drug resistant bacteria, efflux pump inhibitors

INTRODUCTION

The dilemma of antibiotic resistance

The development of antibiotic resistance by microorganisms has established long times before coexistence with mankind [1–3] and constitutes an example of Darwin's concepts of an evolution driven by adaptation to unfavorable environmental conditions [4, 5]. The fact that potent clinically applied antibiotics like penicillin as well as the vast majority of other antimicrobial substances are natural compounds produced by living organisms has created the environmental selective pressure triggering adaptive evolution [2, 4]. In particular plants have evolved a multifaceted repertoire of molecules to combat infections and to protect against herbivore predators [2]. To overcome their vulnerability towards antibiotic compounds in their environments bacteria have developed a multitude of resistance mechanisms which resulted in multiplication and accumulation of antibiotic resistant variants [2]. Besides evolution of enzymes which inactivate antibiotics by molecular destruction or modification, the extraordinary genome plasticity of bacteria in particular lays the basis for their ability to

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adapt rapidly to toxic environments created by antimicrobials [4]. This paves the way for two main principles of achieving resistance: Firstly, by highly effective random mutation, which affects the efficacy of the antibiotic. This may be the case, for example, when i.) the affinity to the antibiotic molecule is lowered, ii.) the drug uptake is decreased, iii.) the drug efflux is increased or, iv.) when distinct metabolic pathways have been affected [4]. Secondly, by complex molecular machineries enabling enormous vertical and horizontal transfers of genetic elements including plasmids, bacteriophages, transposons and large genome fragments such as “pathogenicity islands” [4–6]. Notably, this may occur between bacteria of the same and even different species [6]. Thus, the very limited vertical transfer of resistance properties from “mother to daughter” cells is highly reinforced by horizontal gene transfer between bacteria of the same generation by transformation, transduction or conjugation. Furthermore, distinct genetic information including resistance genes might be shared between commensal (and hence apathogenic), facultative and obligate pathogenic bacteria [7]. Importantly, antibiotic resistance is an ancient evolutionary phenomenon occurring long before and hence, independently from the development of antibiotic substances by humans [7]. Since the discovery of the penicillin by Alexander Fleming in 1928 [1], a multitude of antibiotic substances have been discovered and added to mankind’s armory against bacterial infections. But almost a hundred years later, we still lack potent antimicrobial weapons in order to successfully fight bacterial infections [2]. As multi-drug resistant (MDR) strains are on the rise mostly due to inappropriate use of antibiotics in human and veterinary medicine, finding novel antibiotics-independent ways out of this potentially fatal dilemma becomes a more and more pressing challenge [3, 4]. Many of the currently used antibiotics have been discovered in the 1980s or long before [8, 9]. Despite the emergence of potentially fatal infections with MDR bacteria, particularly in immunocompromised patients, the application of novel antibiotic classes cannot be expected in the near future given that their development has become a financially risky and less lucrative investment for pharmaceutical companies [4, 10]. In 2019, the World Health Organization expressed their concerns that most antibiotics that are currently in the pipeline for future application are being developed with the focus on improvements of already existing antibiotic classes only instead of opening avenues for genuine novel classes [11]. This approach followed by the pharmaceutical industry while being a safer investment from the companies’ and investors’ point of view, makes the newly developed antibiotic substance vulnerable to even faster adaptation by the targeted bacteria as cross-resistance has to be expected [11]. In consequence, tremendous importance comes to finding novel and affordable ways to tackle bacterial infections [12]. One such way might be to survey natural compounds for their antimicrobial properties either alone or in synergy with already established antibiotics.

Capsaicin

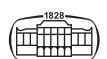
The capsaicinoid capsaicin (8-methyl-*N*-vanillyl-6-non-enamide) is an alkaloid that is produced at variable concentrations by the fruits of the *capsicum* genus (belonging to the *Solanaceae* plant family) and is mostly responsible for the unique feature of almost all peppers including chili peppers, namely their distinct pungency. The extremely hot and burning taste of capsaicin derivatives protects the plants from herbivores, and this feature is responsible for their popularity in large for its usage as spice in gastronomies all over the world. However, capsaicin has also been applied in traditional medicine for topical pain release, weight reduction and as cardioprotective measure, for instance [13, 14]. A recent study revealed the domestication of chili peppers in the Americas more than 6,000 years ago [15]. While being the main capsaicinoid in chili peppers followed by dihydrocapsaicin and nordihydrocapsaicin [16], capsaicin was first partially isolated by Buchholz in 1816 and later named by Thresh in 1876, who had managed to isolate the pure crystalline form [17]. Capsaicin can contribute as much as 1% of the total mass of the pepper fruits and is biosynthesized in the placental epidermis cells near the seeds where it accumulates in so-called “blisters” on the placenta surface [13, 18, 19]. The molecule acts as a strong agonist of the transient receptor potential vanilloid ion-channel receptor 1 (TRPV1) providing its distinct hot, burning taste. However, other beneficial effects such as cardioprotective properties, cannot be accredited to the interaction of capsaicin with the TRPV1 receptor [17]. Remarkably, the neuronal excitation induced by receptor binding is followed by an unusually long refractory period of the neuron, which makes it temporarily insensitive to further (seemingly unrelated) stimuli [14]. This is referred to as desensitization, probably as a result of substance P depletion, and is often exploited in topical creams for capsaicin’s analgesic effect [13, 16]. Upon oral administration, capsaicin is passively absorbed in the stomach and intestine with absorption rates ranging from 50 to 90% [17].

To open novel avenues for fighting bacterial infections in an antibiotics-independent manner, we performed a comprehensive literature survey regarding the antibacterial potential of capsaicin and its derivatives and summarize recent findings regarding i.) direct antibacterial, ii.) antibiotic-resistance-modifying, iii.) bacterial virulence dampening, and iv.) antibiotic pharmacokinetics modulating effects of the molecules.

METHODS

Inclusion and exclusion criteria

All studies conducting *in vitro* or *in vivo* experiments regarding the anti-microbial effects of capsaicin (and its derivatives) have been surveyed, irrespective of the applied experimental model. From these, all publications addressing anti-bacterial properties of capsaicin were included, whereas



papers investigating anti-viral, anti-fungal or anti-parasitic properties were excluded, and hence, are not subject of this survey.

Search strategy

This comprehensive literature survey was conducted applying the MEDLINE database PubMed, where an extensive search was performed between November 2nd and 14th, 2020. The data base was searched for the Mesh terms “Capsaicin” and “Anti-Bacterial Agents” interconnected with the Boolean operator “AND”. From the 36 papers obtained through this query, 18 were *in vitro* studies, 15 *in vivo* reports, two results were reviews and one was available in Chinese language only and therefore had to be prematurely excluded. Of the remaining 33 studies, another 20 had to be excluded due to covering different unrelated topics such as the respiratory system, wound healing, the nervous system and/or simply because they were not bacteria related. This was to be expected by using this rather broadly composed search query and is a welcome circumstance, as it lowers the risk of not including a relevant study. Additionally, some interesting insights about other beneficiary effects of capsaicin were gained through some of the excluded publications and will therefore be part of the discussion. The 13 remaining articles were thoroughly analyzed regarding the compound used, including the extraction method, the different concentrations in terms of the minimum inhibitory concentrations (MICs) and of the tested bacterial strains. Additionally, for *in vivo* trials, the application mode and the applied model including information regarding animal species and human demographics were taken into account. Notably, only the results about capsaicin and its derivatives were included in the following section, even though most of the subsequent studies tested multiple compounds.

RESULTS

Direct antibacterial effects of capsaicin and its derivatives

The investigation of capsaicin and dihydrocapsaicin (DHC) isolated from *Capsicum annum* fruit powder revealed that both molecules exert antimicrobial activity against 16 different strains of clinically relevant MDR Gram-positive and Gram-negative bacteria with varying but high efficacy [20]. Both capsaicin and DHC exerted relatively high MICs of 512 mg/L against most Gram-negative bacteria including *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus* species, while slightly lower MIC values of 256 mg/L were recorded against Gram-positive bacteria such as various methicillin-resistant *Staphylococcus aureus* (MRSA) strains for either compound. The lowest MICs of 128 mg/L were determined for capsaicin against *Bacillus subtilis* and for DHC against *Enterococcus faecalis* (strain ATCC 29523) [20].

In another *in vitro* study, six capsaicin derivatives were synthesized, all of which containing phenolic hydroxyl, a

benzene ring and amide structures, and then tested for their antibacterial effect against *E. coli* and *S. aureus* [21]. The authors reported MICs of the different derivatives ranging from 30 to 250 mg/L against *E. coli* and between 60 and 250 mg/L against *S. aureus*. When assessing the bacterial killing rates (comprising the ratio of bacterial CFUs in the respective verum and the vehicle solution), the capsaicin derivate-induced killing rates of *E. coli* and *S. aureus* ranged from 57.2 to 93.2% and from 60.8 to 96.3%, respectively [21].

In a recent Brazilian study, 2 kg of *Capsicum frutescens* were subjected to solid-phase extraction followed by multiple steps of dilution, filtration and concentration upon evaporation, yielding a stock solution of 10 mg/mL [22]. Additionally, the authors purchased commercial capsaicin and suspended it in 99.9% DMSO to a final concentration of 10 mg/mL. Different Gram-negative bacterial strains (10^8 CFU/mL each) were then co-incubated with 25, 50 and 100 mg/L capsaicin on solid media for up to 24 hours and the bacterial numbers assessed every hour post-incubation. A partial growth inhibition of *Chromobacterium violaceum*, *Serratia marcescens* and *P. aeruginosa* was observed only at the highest dose of either capsaicin preparation (i.e., 100 mg/L). Unexpectedly, co-incubation of *S. marcescens* with 25 and 50 mg/L capsaicin resulted in even enhanced bacterial growth of 46.0 and 38.1%, respectively, indicative for metabolization of the compound. The authors concluded that the MIC had not been reached with the lowest two applied capsaicin concentrations [22].

In further *in vitro* experiments not only the MIC but also the minimum bactericidal concentration (MBC) of capsaicin against *Porphyromonas gingivalis* as example for Gram-negative bacteria which are etiologic agents of periodontal diseases were performed [23]. The MBC was reached when no bacterial growth could be detected within 24 hours of co-incubation. The authors measured an MIC of capsaicin against *P. gingivalis* of 16 mg/L and an MBC of 64 mg/L. Kinetic time-kill studies revealed that *P. gingivalis* growth was inhibited in a capsaicin concentration- and time-dependent manner. Notably, capsaicin concentrations of 16 and 32 mg/L could reduce bacterial biofilm formation by 50 and 90%, respectively [23].

Further evidence for protective effects against dental diseases was provided by experiments applying capsaicin and DHC isolated from an ethyl acetate extract of *Capsicum annum* fruits and additionally including four synthetic capsaicinoid derivatives, namely N-(4-hydroxyphenylethyl) decamide, (E)-N-(4-hydroxy-3-methoxybenzyl)-3,7-dimethylocta-2,6-dienamide, 4-hydroxy-3-methoxy-N-((E)-3,7-dimethylocta-2,6-dienyl)benzamide and N-(4-hydroxy-3-methoxybenzyl)decamide [24]. The determination of respective MICs against *Streptococcus mutans* which causes dental caries revealed that both capsaicin and DHC inhibited growth of the *S. mutans* strain under investigation at 1.25 mg/L while application of the four synthetic derivatives resulted in MICs ranging from 2.5 to 5.0 mg/L [24].

The antibacterial effects of capsaicin and its derivatives were supported by results from experiments with a mixture



comprised of 74.6% capsaicin, 15.8% DHC, 4.4% nordihydrocapsaicin and nonivamide which were analyzed against both Gram-negative and Gram-positive bacteria including *E. coli*, *Pseudomonas solanacearum*, and *Bacillus subtilis*, respectively [25]. Results revealed that the mixture could only hinder the growth of the *E. coli* strain when applied at the highest concentration tested (300 mg/L). Similar effects were obtained for *P. solanacearum*, given that growth was reduced by approximately 20% at the same concentration while the growth of *B. subtilis* was completely inhibited already at the lowest concentration of 25 mg/L [25].

The investigation of the antibacterial activity of different plant-derived extracts and natural compounds against *Pae-nibacillus larvae* which are endospore-forming Gram-positive bacilli causing infectious diseases in honey bees such as American foulbrood [26] showed that of all tested extracts, the compound consisting of 65% capsaicin and 35% DHC was ranking third most effective with a MIC of 32 mg/L [26]. Hence, there is a huge body of evidence for potent antibacterial properties of capsaicin and its derivatives and promising options for their application in human and veterinary medicine as well as in agriculture.

Resistance-modifying properties of capsaicin and its derivatives

The questions if capsaicin might act as an inhibitor of the NorA efflux pump expressed by *S. aureus* contributing to fluoroquinolone resistance and furthermore, reduce intracellular invasiveness of the pathogen, were addressed in a comprehensive study by Kalia and colleagues [27]. Therefore, capsaicin was analyzed for its potential to reduce the MIC of ciprofloxacin against the NorA overproducing *S. aureus* SA-1199B strain, against the parental (wild-type) strain *S. aureus* SA-1199 and against the NorA knockout strain *S. aureus* SA-K1758. Co-incubation with 25 mg/L capsaicin resulted in a 2- and 4-times reduction of the ciprofloxacin MIC against the NorA overproducing *S. aureus* SA-1199B strain and the *S. aureus* SA-1199 wild-type strain, respectively, which was equivalent to the reduction achieved with the same concentration of the efflux pump inhibitor reserpine. Doubling of the capsaicin concentration (i.e., 50 mg/L) did not further decrease the ciprofloxacin MIC. Notably, neither capsaicin nor reserpine resulted in a change of the ciprofloxacin MIC against *S. aureus* SA-K1758 (i.e., NorA knockout) strain. Furthermore, the authors performed a time-kill assay against the NorA overproducing *S. aureus* SA-1199B strain in order to quantify the bactericidal properties of the combination of capsaicin and ciprofloxacin. Ciprofloxacin was first tested at a quarter of its MIC but did not cause any bacterial growth inhibition. When adding capsaicin (25 mg/L), however, a bactericidal activity could be assessed that was comparable to that observed with ciprofloxacin at its MIC. Furthermore, capsaicin (25 mg/L) was able to elongate the post-antibiotic effect of ciprofloxacin up to more than three times (i.e., 0.3–1 hour at $0.25 \times$ MIC of ciprofloxacin). Capsaicin was also further examined for its potential to reduce the ethidium bromide efflux in the NorA

overproducing *S. aureus* SA-1199B strain applying a fluorescence assay. In fact, 25 mg/L capsaicin could increase the intracellular amount of ethidium bromide by about five times as compared to the negative control indicative for a strong potency for impeding the ethidium bromide efflux. Finally, 25 mg/L capsaicin was analyzed for its ability to reduce invasion of macrophages by *S. aureus* SA-1199B (NorA overproducing strain) and *S. aureus* SA-1199 (wild-type strains). The results revealed that capsaicin was indeed capable of reducing the invasiveness of the two strains, with the former being hampered more effectively as compared to the latter [27].

A recent comprehensive study investigated the antibacterial, resistance-modulatory and efflux pump inhibitory properties of several capsaicinoids and capsinoids against Mycobacteria such as *Mycobacterium smegmatis* [28]. Whereas the two natural compounds capsaicin and DHC had been purchased, the remaining compounds were synthesized in-house. The authors measured MICs of >128 mg/L for respective compounds applied alone indicative for a lack of antimycobacterial effect. For addressing potential resistance-modifying properties, respective compounds were applied at half of their respective MIC in combination with rifampicin or with ethidium bromide constituting a well-known substrate for efflux pumps. In case of DHC, modulation factors of 8 and 32 could be assessed when combined with rifampicin and ethidium bromide, respectively. This indicates that when applied together with DHC, only 1/8 and 1/32 of the rifampicin and ethidium bromide concentrations are needed, respectively, in order to achieve the same antimycobacterial effects as if treated with respective compound alone. Furthermore, a fractional inhibitory concentration index of 0.38 could be determined for DHC and rifampicin indicative for strong synergistic effects directed against *M. smegmatis*. In conclusion, the authors proposed potent efflux pump inhibitory effects of capsaicin and DHC as resistance-modifying mechanisms in Mycobacteria [28].

Capsaicin and modulating effects on antibiotic pharmacokinetics

The following three included studies [29–31] did not investigate direct antimicrobial properties of capsaicin and its derivatives but instead, conducted *in vivo* experiments on the effects of capsaicin on the intestinal absorption and thus, bioavailability of distinct antibiotic compounds such as the fluoroquinolone ciprofloxacin and the cephalosporins cephalexin and cephazolin.

In a study from Mexico [29], the authors addressed whether co-administration of capsaicin and ciprofloxacin could impact antibiotic bioavailability of the latter. Therefore, Wistar rats were randomly divided into seven groups and perorally subjected to ciprofloxacin plus capsaicin in concentrations ranging from 0.01 to 1.0% versus placebo. Rats from the 0.5% capsaicin treatment group exhibited 72% higher maximum ciprofloxacin plasma concentrations as compared to the placebo and ciprofloxacin-treated control animals. In the 1.0% capsaicin cohort, however, the

maximum ciprofloxacin plasma levels were lower as compared to those measured in the rats that had received half the capsaicin dose (i.e., 0.5%). The authors concluded that capsaicin promotes ciprofloxacin peak plasma levels in a dose-independent manner, but does neither impact the reabsorption nor the clearance rates of the fluorochinolone [29].

In another study the upper part of the jejunum and the lower part of the ileum were cannulated in Wistar rats in order to perfuse the cannulated segments with a solution containing cephalixin and capsaicin at a concentration of 10 or 400 μM [30]. Unexpectedly, assessment of the absorption rates revealed that 400 μM capsaicin decreased cephalixin absorption from 1.16 to 0.54 nmol/min/cm in the jejunum and from 0.9 to 0.46 nmol/min/cm in the ileum [30].

In contrast, when testing the impact of capsaicin on the small intestinal concentration of a different cephalosporin, namely cephazolin, applying the same experimental model, increases of the cephazolin concentrations within the rat jejunum from 15.3 to 22.8 and 23.4 $\mu\text{g}/\text{cm}$ following application of 10 and 100 μM , respectively, were observed [31]. Hence, capsaicin might impact the bioavailability of distinct antibiotic compounds.

Virulence-attenuating effects of capsaicin

A previous *in vitro* study assessed the anti-pathogenic potential of different natural compounds including red chili and capsaicin against *Vibrio cholerae* El Tor [32]. After incubation of four different pathogenic strains with red chili extract (100 mg/L), the cholera toxin concentrations were measured in the culture supernatants. Remarkably, co-incubation with the red chili extract resulted in a more than 80% reduction of the cholera toxin secretion. The authors then introduced active ingredients of the red chili extract including capsaicin into their survey. Upon coincubation with 23 *V. cholerae* strains of different serogroups and cholera toxin genotypes, capsaicin could effectively suppress cholera toxin production in a dose-dependent manner without affecting bacterial growth, however. Further molecular analyses revealed that red chili extract and capsaicin decreased the transcription levels of *ctxA*, one of the key virulence genes of *V. cholerae* El Tor, by 43 and 23 times, respectively. In addition, capsaicin repressed transcription of distinct virulence genes such as *tcpA* (6.3 times), *toxT* (4.0 times), *tcpP* (2.7 times) and *tcpH* (2.5 times) involved in bacterial toxin production whereas the transcription of *hns*, a gene regulator that is known to suppress the transcription of several virulence genes, was found to be enhanced by more than two times [32]. Hence, capsaicin could effectively dampen expression of distinct key virulence genes in *V. cholerae* El Tor.

Disease-ameliorating effects of capsaicin

Toyoda and coworkers investigated the efficacy of >98% pure capsaicin in the treatment of *Helicobacter pylori* induced gastritis both *in vitro* and *in vivo* [33]. Therefore, a *H. pylori* strain was grown in Brucella broth containing

capsaicin in different concentrations (namely, 1 μM , 10 μM , 100 μM). The inhibitory effects were assessed by enumeration of the colony forming units (CFUs), with most prominent effects exerted by the highest applied capsaicin concentration (i.e., 100 μM , $P < 0.05$). In an *in vivo* experiment, Mongolian gerbils were perorally infected with *H. pylori* and either fed a diet with or without capsaicin. In the gerbils from the capsaicin cohort, not only less distinct neutrophilic infiltration of the gastric antrum and corpus, but also less pronounced formation of heterotopic proliferative glands in the corpus could be observed as compared to the control group indicative for less pathogen induced inflammatory sequelae upon capsaicin application [33]. Hence, dietary capsaicin application exerted anti-inflammatory effects in experimental *H. pylori* induced chronic gastritis.

Further effects and applications of capsaicin

When compared to studies addressing potential antimicrobial effects of capsaicin and its derivatives, far more research has been undertaken to date regarding the health-promoting effects of the natural compound as topical analgesic or as adjunct treatment option against a multitude of different cancer types including colon [34], pancreatic [35], hepatocellular [36], prostate [37] or small cell lung cancer [38]. Overall, the obtained results are rather inconclusive and conflicting, however, and strongly depend on the distinct cancer type, the location and the stage of the disease. Furthermore, even dose-dependent carcinogenesis-promoting effect of capsaicin have been described, given enhanced cell proliferation, invasiveness, migration, and ultimately metastasis in the presence of even relatively low phytochemical concentrations in some cancer types [39–41]. There is furthermore evidence suggesting capsaicin as a promising (adjunct) option for the therapy and/or prevention of pain syndromes, mainly in topical application [42], of obesity and metabolic syndrome [43, 44], and of myocardial injury [45].

DISCUSSION

Findings of the literature research

Overall, capsaicin and its derivatives have been shown to exert both direct and indirect antibacterial effects which are, however, highly depending on many factors such as the bacterial species and strains, the experimental models and settings, and the respective compounds and applied doses. For instance, a capsaicin derivative was found to be an effective inhibitor of *E. coli* with an MIC as low as 31.3 mg/L [21], while two other studies using capsaicin itself, identified rather poor MICs of >300 mg/L [20, 25], further underlining that capsaicin on the one hand side and its derivatives on the other may exert divergent antimicrobial properties. Other Gram-negative bacteria such as *H. pylori* and *P. gingivalis* appeared to be quite susceptible to capsaicin [23, 32], whereas the growth of different strains of *C. violaceum* as



well as of *S. marcescens* and *P. aeruginosa* were only moderately inhibited by the compounds tested [22]. Furthermore, *K. pneumoniae*, *P. solanacearum*, *Proteus* species and a different *P. aeruginosa* strain, which showed moderate sensitivity in another analysis [22], could not be inhibited by the capsaicin compound used in respective studies [20, 25]. Of the Gram-positive bacterial strains under investigation only four, namely *S. aureus*, *B. subtilis*, *S. mutans*, and *P. larvae* showed significant susceptibility to capsaicin or its derivatives [21, 24–26]. Only little or even no growth inhibition of *E. faecalis* and *M. smegmatis* as well as of various MRSA strains was achieved by the capsaicinoids and capsinoids under investigation [20, 23, 28]. However, capsaicin was shown to inhibit activities of distinct efflux pumps which contribute to the MDR features of MRSA, for instance [46]. Notably, co-administration of capsaicin which had been identified as a novel inhibitor of the NorA efflux pump contributing to ciprofloxacin resistance, resulted in a pronounced decrease of the ciprofloxacin MIC against *S. aureus*, extended the post-antibiotic effect of the fluoroquinolone and reduced the invasiveness of the pathogen and hence, its virulence *in vitro* [27]. In support, both capsaicin and DHC exerted remarkable efflux pump inhibiting activities in *M. smegmatis* [28]. Furthermore, strong synergistic antibiotic effects of DHC and rifampicin could be shown against *M. smegmatis in vitro* [28]. Thus, co-administration of distinct antibiotics and capsaicin or its derivatives may yield synergistic antimicrobial effects even in MDR bacterial infections [20, 27, 28].

Apart from direct antibacterial properties, capsaicin was shown to exert indirect disease-alleviating effects during bacterial infection, given that capsaicin dampened anti-inflammatory responses during *H. pylori* induced gastritis by suppressing tumor necrosis factor-alpha (TNF-alpha) expression, for instance [33]. Moreover, capsaicin enhanced the intestinal absorption of ciprofloxacin and of cephazolin [29], which was, however, not the case when applying another cephalosporin, namely cephalexin, in the same experimental setup in the framework of another study [30, 31]. Remarkably, capsaicin acted as a potent inhibitor of cholera toxin production in multiple different strains and serotypes of *V. cholerae* El Tor, probably by *H-NS* mediated inhibition of the transcription of major virulence genes, whereas pathogenic growth was unaffected [32]. The virulence alleviating effects of capsaicin were further supported by less invasive properties of *S. aureus* after capsaicin application *in vitro* [27].

Conclusion and outlook

Our comprehensive literature research provides evidence that capsaicin and its derivatives constitute promising adjunct antibiotics-independent options to treat bacterial infections that are even caused by MDR strains. Particularly the synergistic effects of capsaicin observed in combination with established antibiotics against distinct pathogens *in vitro* should encourage to perform further studies in order to shed more lights onto the underlying molecular mechanisms and, even more importantly, to substantiate the *in vitro* findings in

in vivo models and subsequent clinical trials. Capsaicin might be not potent enough to replace already existing antibiotics, but its capabilities in lowering the amount of the latter when treating bacterial infections will contribute to reduce the risk of resistance development and of side effects experienced by the patient. Furthermore, future in-depth investigations on combinations of different natural compounds with or without antibiotics regarding their antibacterial, resistance-modifying and immune-modulatory properties might be important corner stones in the combat of (MDR) bacterial infections in human and veterinary medicine [47, 48].

Limitations

One limitation of this survey is the relatively small number of included publications. The search strategy was performed as thoroughly as possible and the papers evaluated carefully. Given that the quality assessment of the publications was performed by a single investigator only, mistakes cannot be excluded, and relevant studies might not have been considered. With regard to the included publications, a main limitation of this survey is the lack of comparability between studies due to main differences in experimental set-ups including applied compounds, solubilizer, concentrations and bacterial strains, for instance, leading to conflicting results. Furthermore, the vast majority of the assessed data have been derived from *in vitro* experiments, whereas of the 13 included studies only four had been performed *in vivo*, of which none included human subjects. Hence, one should be very careful when drawing definite conclusions.

Ethics statement: Not applicable (literature survey).

Funding: This work was supported by grants from the German Federal Ministries of Education and Research (BMBF) in frame of the zoonoses research consortium PAC-Campylobacter (IP7/01KI1725D) and from the Federal Ministry for Economic Affairs and Energy following a resolution of the German National Parliament, Deutscher Bundestag (ZIM, ZF4117908 AJ8) to SB and MMH.

The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Authors' contribution: SF conceived and designed the survey, wrote the paper. SM edited paper. SB provided critical advice in design of the survey, edited paper. MMH supervised the survey, co-wrote the paper.

Conflict of interest: SB and MMH are Editorial Board members.

LIST OF ABBREVIATIONS

CFU	colony forming units
DHC	dihydrocapsaicin
DMSO	dimethyl sulfoxide
MBC	minimum bactericidal concentration
MDR	multi-drug resistant



MIC minimum inhibitory concentration
 MRSA methicillin-resistant *Staphylococcus aureus*
 TNF tumor necrosis factor

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