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Review

Biocide resistance in *Klebsiella pneumoniae*: a narrative review

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SUMMARY

Klebsiella pneumoniae is among the World Health Organization's list of priority pathogens, notorious for its role in causing healthcare-associated infections and neonatal sepsis globally. Containment of *K. pneumoniae* transmission depends on the continued effectiveness of antimicrobials and of biocides used for topical antisepsis and surface disinfection. *Klebsiella pneumoniae* is known to disseminate antimicrobial resistance (AMR) through a large auxiliary genome made up of plasmids, transposons and integrons, enabling it to evade antimicrobial killing through the use of efflux systems and biofilm development. Because AMR mechanisms are also known to impart tolerance to biocides, AMR is frequently linked with biocide resistance (BR). However, despite extensive research on AMR, there is a gap in knowledge about BR and the extent to which AMR and BR mechanisms overlap remains debatable. The aim of this paper is to review and summarise the current knowledge on the determinants of BR in *K. pneumoniae* and highlight content areas that require further inquiry.

Introduction

Klebsiella pneumoniae, first described by Carl Friedlaender in 1882 [1], is an opportunistic pathogen naturally existing as part of the human gut microbiome. *Klebsiella pneumoniae* has gained notoriety in recent decades as a highly virulent nosocomial pathogen increasingly resistant to first- and second-line antimicrobial therapy and has been recognised as the leading cause of neonatal sepsis in low- and middle-income countries, associated with a case fatality rate of up to 30% [2,3]. It is among a group of organisms often associated with antimicrobial resistance (AMR), collectively called ESKAPE, an acronym for *Enterobacter* spp., *Staphylococcus aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas*

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aeruginosa and *Enterococcus faecium* [4]. *Klebsiella pneumoniae* has a substantial accessory genome of plasmids and chromosomal gene loci, which can be used to classify it into classical, highly virulent and/or multidrug-resistant *K. pneumoniae*. There is evidence to suggest that classical *K. pneumoniae* strains are increasingly being replaced by 'highly virulent *K. pneumoniae*' which is known to cause serious infections in even immunocompetent populations [6,5]. These accessory genomes enable *K. pneumoniae* to evade the action of several antimicrobial drugs by coding for enzymes and other cellular components that enable this bacterium to withstand antibiotic actions. The resistance of *K. pneumoniae* to amikacin, gentamicin and third generation cephalosporins severely limits the treatment options in resistant-*K. pneumoniae* infections, and the recent development of resistance to carbapenems exacerbates the problem [7].

Transmission of *K. pneumoniae* among hospitalized patients is believed to occur through direct and indirect contact in the healthcare environment. Hospital infection prevention protocols play a major role in containing the spread of AMR and rely on the consistent efficacy of biocides, a group of chemicals used as topical antiseptics and surface disinfectants [8]. A wide variety of biocides are used to disinfect surfaces and equipment in the hospital environment, including alcohols, chlorine-releasing agents, quaternary ammonium compounds, orthophthalaldehyde and phenolics. Although the concentrations used in the clinical context are usually higher than required to inhibit the organisms, variables such as organic matter, biofilm formation and exposure time may interfere with activity of the biocides [9,10]. Following the paradigm of AMR, concern has been raised that sub-lethal doses of biocide might select for biocide resistance (BR), leading to environmental persistence of pathogens, especially those known for harbouring AMR. Understanding the extent to which BR influences environmental persistence of pathogens, and the modifiable factors responsible for propagating BR, is critical in preventing and containing nosocomial infections.

Mechanisms of BR in K. pneumoniae

In this review, BR is defined as the ability of bacteria to grow in the presence of high concentrations of biocides, regardless of the exposure time. This is a trait that is usually associated with active defence against biocides and is conferred via mutations. Biocide tolerance, on the other hand, is characterized as the ability to withstand temporary exposure to high concentrations of biocides, which is typically achieved by decelerating important biological processes. The mechanisms of BR are similar to those of AMR: limited uptake, limitation of biocide accumulation as a function of enhanced antiseptic efflux, and (although rare) target site mutations [11]. Biocide resistance may be an intrinsic property of bacteria, or it may arise because of a mutation or amplification of an endogenous chromosomal gene, or by acquisition from plasmids, transposons and integrons [12–14]. Biocide resistance occurs commonly as a result of cell wall changes that reduce permeability or enhance biocide efflux [15]. Biocide resistance has been recorded as far back as 1945 for phenol-resistant microbes in human faeces [16] and has been described in *Enterococcus* spp., *S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa* and *Enterobacter* spp. Both low- and high-level resistance to triclosan, quaternary ammonium compounds and chlorohexidine have been found in *S. aureus* [17,18] and *P. aeruginosa* [19,20].

Bacterial envelope changes have been described as the most common mechanism of resistance especially in biofilm-forming Gram-negative bacteria [15]. Gram-negative bacteria possess a complex matrix of membranes, efflux proteins and a peptidoglycan layer that impair passage of biocides into the cells. Cellular structure changes, such as altering expression of hydrophilic porin channels, confer resistance to hydrophilic agents such as quaternary ammonium compounds and chlorhexidine gluconate [21]. These bacteria are also able to form biofilms, in which sessile communities of bacteria embedded in an extracellular matrix show altered growth. In this context, resistance is due to several different mechanisms developed by the bacterial biofilm. These include: the failure of biocides to bind and penetrate the biofilm, the neutralisation of biocides by proteins in the biofilm, and the altered growth of bacterial cells because of nutrient depletion in the biofilm [22].

Although the mechanisms of BR in *K. pneumoniae* have not been fully elucidated, it is clear that biofilms play an integral role in antiseptic resistance, which complements with the efflux systems and other mechanisms [11]. Biofilms in *K. pneumoniae* have been associated with peracetic acid resistance in carbapenem-resistant *K. pneumoniae* (CRKP) [23]. In addition, a study performed by Betchen *et al.* [24] on one of the ESKAPE pathogens, *A. baumannii*, showed that when biofilm formation occurred, a noticeable reduction in disinfectant effectiveness was observed. This may suggest that biocide tolerance could be due to biofilm formation rather than the expression of antiseptic-resistance genes [24]. It should be taken into consideration that biofilms are heterogeneous in nature and may not have the same biocide tolerance/resistance effect.

Likewise, the role of efflux systems is unclear (Table I). These systems are capable of transporting structurally different compounds thereby conferring co-resistance to different classes of biocides and even antibiotics [25,26]. Such systems include: the olaquindox/quinolone efflux pumps, encoded by the *oqxA* and *oqxB* genes, which belong to the resistance-nodulation cell division (RND) family; and the quaternary-ammonium-compounds resistance determinant, *qacE*, along with its active deletion derivative, *qacEΔ1*, which belong to the small multi-drug resistance (SMR) family. It is important to note that since these efflux pumps may also accommodate antimicrobial drugs, there is the concern of antimicrobial drug/biocide cross-resistance [27].

AMR and BR in K. pneumoniae: a health-care concern

The emergence of BR in multi-drug resistant *K. pneumoniae* (MDRKP) and CRKP presents a challenge in containing nosocomial infections. Several studies have associated MDRKP and CRKP with BR, suggesting that there is circulation of 'super-*K. pneumoniae*' which is able to resist both biocide and antibiotic pressure. It is known that there are genetic variations in *K. pneumoniae* that confer both AMR and BR [28]. As an example, we highlight the presence of OqxAB efflux pump which transports tigecycline, quinolones, quaternary ammonium compounds and biguanides, in Enterobacterales including *K. pneumoniae* [29].

Morante *et al.* [30] noted a positive association between chlorhexidine resistance and resistance to trimethoprim-

Table I
List of studies showing the role of efflux pumps to biocide/antiseptic tolerance in *K. pneumoniae*, 2002–2022

Citation	Study	Conclusion
Fang *et al.* [67]	Cloning of a cation efflux pump gene associated with Chlorhexidine resistance in *K. pneumoniae*.	*CepA* is associated with Chlorhexidine resistance and may act as a cation efflux pump.
Abuzaid *et al.* [45]	*Klebsiella pneumoniae* susceptibility to biocides and its association with *cepA*, *qacΔE1* and *qacE* efflux pump genes and antibiotic resistance.	There was a close link between carriage of efflux pump genes, *cepA*, *qacΔE1* and *qacE* genes and reduced biocide susceptibility, but not antibiotic resistance, in *K. pneumoniae* clinical isolates.
Napasterk *et al.* [39]	Reduced susceptibility to chlorhexidine in extremely-drug-resistant strains of *Klebsiella pneumoniae*.	Reduced susceptibility to chlorhexidine appeared to be independent of the expression of *cepA*, *acrA* and *kdeA* efflux pumps.
Azadpour *et al.* [49]	Prescence of *cepA* and *qacED1* genes and susceptibility to hospital biocides in clinical isolates of *K. pneumoniae* in Iran.	No significant association of biocide resistance and *cepA* and *qacED1* was observed, rather a close association between *qacED1* and antibiotic resistance.
Guo *et al.* [68]	Determining the resistance of Carbapenem resistant *K. pneumoniae* to common disinfectants and elucidating the underlying mechanisms	The pan-resistant CRKP contained various MDR genes (*qacA*, *qacE*, *qacED1*, *acrA*) and exhibited resistance to ethyl-alcohol, iodophor and chlorhexidine acetate.
Vijaykumar *et al.* [48]	Distribution of biocide resistance genes and biocides susceptibility in multi-drug resistant *K. pneumoniae*, *P. aeruginosa and A. baumannii*	No significant correlation between presence or absence of biocide resistance genes and MIC observed.
Wand *et al.* [55]	*SmvA* is an important efflux pump for cationic biocides in *K. pneumoniae* and other Enterobacteriaceae	Increased expression of *SmvA* results in increased Chlorhexidine resistance. Also, loss of the *smvA* regulator, *SmvR* results in increased *smvA* expression and, consequently, increased chlorhexidine resistance.
Gual-de-Torrella *et al.* [40]	In vitro activity of six biocides against Carbapenemase-producing *K. pneumoniae* and presence of genes encoding efflux pumps	The activity of some biocides is affected by temperature and growth media. This activity, in terms of MICs, are not related to the type of clone, ST, Carbapenemase or the presence of the efflux pump genes.
Wand *et al.* [38]	Contribution of the efflux pump AcrAB-TolC to the tolerance of chlorhexidine and other biocides in *Klebsiella spp*	Biocide tolerance in *K. pneumoniae* is dependent upon several components, with increased efflux through *AcrAB-TolC* being an important one.
Ni *et al.* [52]	Disinfection strategies for Carbapenem-resistant *K.pneumoniae in a Healthcare facility*.	The CRKP strains showed extensive resistance to clinically used disinfectants, with high efflux pump gene carrier rates.

sulfamethoxazole in clinically related *K. pneumoniae* carrying the bla_{NDM} gene and suggested that the presence of carbapenemases is directly associated with high chlorhexidine Minimum Inhibitory Concentrations (MICs), and therefore tolerance to this biocide. This is a similar finding to that of Koljalg *et al.* [31] and Denkel *et al.* [32], who suggested that AMR is a known indicator of chlorhexidine susceptibility. In the latter study, high chlorhexidine MICs were associated with longer hospitalization at the intensive care unit and extended antibiotic therapy. This is a phenomenon associated with some and not all antiseptics and antibiotics. For example, Morante *et al.* [30] found no relationship between AMR and alcohol tolerance. This is because the mode of action of alcohols are not related to the mechanisms of antibiotic's resistance, and so on they do not select for resistant variants in bacteria.

Although BR has been recorded dating as far back as 1940s [16], there exists an information vacuum about BR in *K. pneumoniae* especially in low and middle-income countries [33].

It is important to highlight that the association between MDR and BR is debateable, with some authors suggesting that there is little evidence that MDR bacterial strains have elevated resistance levels to biocides compared to more susceptible isolates, or that biocide exposure, in general, selects for MDR bacteria over more susceptible strains [34].

The aim of this paper is to review the available literature on the determinants of BR in *K. pneumoniae* and highlight areas that require further inquiry.

Methods

Selection of articles

The aim of this narrative literature review is to provide an overview of BR in *K. pneumoniae*. Medline, EMBASE and Web of Science were consulted for primary information on the topic. Furthermore, Google Scholar was also consulted for completeness. All publications that matched "antiseptic tolerance *Klebsiella pneumoniae*", "antiseptic resistance *Klebsiella pneumoniae*", "biocide resistance *Klebsiella pneumoniae*" and "biocide tolerance *Klebsiella pneumoniae*" were included. The titles of the references were scanned for

keywords matching our selection criteria and included if they met at least one of them. Two authors (PN and GMP) independently reviewed the titles, abstracts and full articles of the retrieved papers.

Inclusion and exclusion criteria

Only studies that discussed antiseptic/biocide/disinfectant resistance in *K. pneumoniae* were included. The inclusion criteria were restricted to papers published in English language until August 2023.

Results

This review focuses on the role of different mechanisms of resistance and their role in BR in *K. pneumoniae*. Twenty-four (24) papers were retrieved for this review and publications from all around the world were included. Nevertheless, to achieve a comprehensive picture, we also addressed their role in AMR.

Multi-drug efflux pumps

AcrAB-TolC

Acriflavine resistance component A and B (AcrAB-TolC) is a multi-drug efflux pump belonging to the RND family present in Enterobacterales conferring resistance to several compounds, including acriflavine, a topical antiseptic [35]. It is encoded by the *acrRAB* operon in which *acrR* codes for the repressor of the operon gene, and *acrA* and *acrB* code for periplasmic lipoproteins that connect with the envelope protein TolC. The expression of *AcrAB-TolC* is primarily regulated by the transcriptional activator *ramA* [36]. RamA *expression is controlled by* ramR, *which represses the activation of* ramA [36]. Mutations in *ramR* and exposure to sub-lethal concentrations to benzalkonium chloride, triclosan and chlorhexidine have been found to increase expression of *ramA*, the *acrA* activator and thereby conferring biocide tolerance and multi-drug resistance [37,38].

Despite this, Naparsterk *et al*. [39] found no association between resistance to chlorhexidine and expression of *acrA* in extremely-drug resistant *K. pneumoniae*. The author concluded that the phenomenon of chlorhexidine resistance may not be rendered genetically. In 2021, Gual-de-Torella *et al*. [40] arrived at the same conclusion highlighting that biocide tolerance was independent of presence/absence of efflux pump genes including *acrAB*, sequence or clone types. Similarly Samir *et al*. . [41] found no linkage between the presence of integrons, which usually carry biocide resistance genes, and biocide resistance in multi-drug resistant *Klebsiella pneumoniae*.

Wand *et al*. [38] characterised NCTC7427, an ST86 strain (a hypervirulent *K. pneumoniae* strain) with inactive AcrAB-TolC. The strain harboured DNA sequences of *SmvA*, *oqxAB* and *CepA* and their regulators, but showed more than 4-fold increase in susceptibility to benzalkonium chloride, chlorhexidine and triclosan when compared to KPUK02, another ST86 strain. NCTC7427 did not exhibit any increase in susceptibility to sodium hypochlorite, glutaraldehyde and silver nitrate, suggesting that: *a*) the phenotype showed was due to presence/absence of AcrAB and *b*) the pump is crucial to tolerance of some biocides and not others. When comparing six ST258 strains (NTCT 13438, 46704, CFI_131_KPC2, CFI_141_KPC3, CFI_147_KPC2 and MKP103), MKP103 showed a consistently elevated MIC (usually>4-fold) for several biocides including cetrimide and chlorhexidine. The elevated MIC was associated with elevated expression of *acrA* and *ramA* in the MKP103 strain as compared to other ST258 strains.

Importantly, the AcrAB-TolC pump has been also implicated in aminoglycoside, tetracycline and fluroquinolone resistance [42,43], leading to the question of antimicrobial/biocide cross-resistance.

CepA

The Cation Efflux Pump, denoted CepA is a putative efflux system common in Enterobacterales that mediates chlorhexidine resistance [44]. Abuzaid *et al*. [45] found an association between CepA and chlorhexidine resistance. The MICs performed in the presence of an efflux pump inhibitor showed a significant decrease which was customarily associated with CepA. This finding is similar to Mendes *et al*. [46] in which there was a 4-fold decrease in the MIC for chlorhexidine when carbonyl cyanide m-chlorophenyl hydrazone (CCCP), an efflux pump inhibitor, was added, in CepA-carrying *K. pneumoniae* isolates. There was a close association between carriage of efflux pumps, including CepA, and BR.

Despite the demonstration of the effect of efflux-pump inhibitors in previous studies, Shohreh *et al*. [47] found no association between resistance to benzalkonium chloride and *CepA* gene in *K. pneumoniae*, although there was an association with chlorhexidine tolerance. Vijaykumar *et al*. [48] found no association between the presence of BR genes, including *CepA* with multi-drug resistant phenotype and/or BR, and these findings are similar to those of Naparsterk *et al*. [39] who found no significant association between susceptibility to chlorhexidine and *CepA* expression in extensively drug-resistant *K. pneumoniae*. Azadpour *et al*., [49] also noted that there was no association between presence of *CepA* and AMR and/or BR.

OqxAB

OqxAB efflux pump is an RND-type efflux pump carried by the pOLA52 plasmid and encoded by *oqxA* and *oqxB* genes. It has been mostly associated with fluoroquinolone resistance but also resistance to chlorhexidine, triclosan and ethidium bromide [50,51]. Ni *et al*. [52] found out that the MIC and Minimum Bactericidal Concentration (MBC) of 3% hydrogen peroxide, showed a statistical difference between the negative and positive *oqxA* gene groups, suggesting that the pump may be instrumental in hydrogen peroxide resistance. *OqxAB* was also demonstrated to offer resistance to norfloxacin, sodium-dodecyl sulphate and ethidium bromide. Findings from Ni *et al*. [53] showed that, after a susceptible ATCC10031 strain was used to select for drug-resistant mutants in the presence of selected antibiotics, an oxacillin resistant variant, OX128, overexpressed *oqxAB* and also showed increased tolerance to cloxacillin, norfloxacin, sodium dodecyl sulphate (>2-fold), rhodamine 6G (8-fold), acriflavine (4-fold), benzalkonium chloride (4-fold) and ethidium bromide (4-fold). These results also suggest that ß-lactam antibiotics may be substrates for oqxAB/RND-mediated efflux [43,52].

qacE, qacEΔ1

Quaternary ammonium compound efflux systems (*qac*) are plasmid-borne genes belonging to the SMR family. These efflux pumps offer adaptive response to lipophilic and cationic compounds [49]. Although they are named after quaternary ammonium compounds, they transport a wide variety of structurally different molecules and can therefore offer co-resistance to biocides and antimicrobials [27]. The *qac* genes are classified into *qac A/B, C/D, E/F, G, H, J* and *Z*, with *qacE* and its variant, *qacEΔ1*, being prevalent in Enterobacterales and Gram-negative bacteria [54].

In 2012, Abuzaid *et al*. [45] found a close association between carriage of *qacE* and *qacEΔ1* (along with *CepA*) and reduced susceptibility to antiseptics but not AMR. However, the role of these efflux pumps was partial, since isolates with and without these antiseptic resistance genes exhibited high MICs for chlorhexidine, triGene and benzalkonium chloride. Upon addition of CCCP, there was a correlation between a reduction of MICs for chlorhexidine and medihex-4, and the presence of *qacEΔ1*. Ni *et al*. [52] also concluded that *qacEΔ1* has a role in resistance to quaternary ammonium compounds. Only the MIC of 0.1% benzalkonium bromide showed statistical difference between the group that harboured the *qacEΔ1* gene and the group that did not [52].

This completely contradicts the findings of Azadpour *et al*. [49] in which a close association between *qacEΔ1* gene and antibiotic resistance was found, but no significant association of BR with the presence of *qacEΔ1* and *CepA* in clinical *K. pneumoniae* isolates.

In contrast, Vijayakumar *et al*. [48] found no significant association between the carriage or absence of antiseptic resistance genes in multi-drug resistant *K. pneumoniae* and resistance to cetrimide, benzalkonium chloride and chlorhexidine gluconate. Furthermore, although most of the isolates were resistant to cefepime, ceftazidime, gentamicin, amikacin, tobramycin, piperacillin and carbapenem groups, the presence/absence of *qacE* or its attenuated variant could not be ascertained as the cause [48].

SmvA/SmvR

SmvA is a chromosomally encoded efflux pump of the Major Facilitator Superfamily, a membrane transport protein. Deletions in the regulator, *SmvR*, have been associated with increased expression of *SmvA* and thereby increased chlorhexidine tolerance. In a study performed by Wand *et al*. [55], it was found out that: *a*) Enterobacterales without *SmvR* were less susceptible (≥2-fold) to chlorhexidine as compared to strain MKP103 which carried both *SmvA* and *SmvR*. For strains carrying Δ*smvA*, susceptibility increased 2-fold for chlorhexidine, cetrimide, cetyltrimethylammonium bromide and hexadecylpyridinium chloride monohydrate; *b*) the loss of function of *SmvR*, through adaptation to chlorhexidine, influenced tolerance to other cationic biocides, a phenomenon that can lead to cross-resistance to biocides. Although the mutations are a risk for the development of biocide cross-resistance, they did not have any effect on AMR in the MKP103 strain.

These efflux pump can transport a wide variety of biocides as summarised in Table II.

Biofilm formation

Biofilms are communities of bacteria, attached to a surface and characterized by an extracellular matrix, with an increased antibiotic and biocidal resistance and tolerance to desiccation [56]. It is well known that the extensive use of biocides in the environment induces cross-resistance to other biocides and antibiotics and can increase the ability of bacteria to form biofilms [57]. *Klebsiella pneumoniae* is able to generate a thick layer of biofilm as one of its virulence factors' repertoire. A majority of biofilm is made up of extra cellular polymers that offer protection against oxidative stress, harsh environmental conditions and also biocides [56].

It has been shown that chlorine concentrations within the biofilms of *K. pneumoniae* were only 20% of the concentrations in the working biocide. This may demonstrate the effect of biofilms on chlorine-releasing agents [58]. Further, Jang *et al*. [59] also demonstrated that chlorine at a concentration of 25mg/l did not penetrate more than 100μm in biofilms that are 150—200μm thick. Due to interactions of the biocide with the protein matter in the biofilm, the concentrations are diluted even further, leading to sublethal concentrations in the biofilm and eventually to the possible development of biocide tolerance. Brunke *et al*. [23] also characterised an ST101 oxacillinase-48 (*OXA-48*) *Carbapenemase-producing* K. pneumoniae (OXA-48-Kp) that was responsible for an outbreak. The results showed a marked decrease sensitivity to peracetic acid after production of a biofilm. These results were consistent with those of another ST101 strain, another carbapenemase-producing *K. pneumoniae*, showing that CRKP was able to withstand reprocessing with peracetic acid [60].

In a study to determine the role of biofilms on glutaraldehyde tolerance, Cholley *et al*. [61] concluded that *K. pneumoniae* was able to persist and even regrow after exposure to 2% (working concentration) and 1% glutaraldehyde,

Table II
Summary of multi-drug efflux pumps genes, their known variants, and their substrates

Gene	Gene variation(s)	Substrates
AcrAB-TolC	-	Benzalkonium chloride [37]; Chlorhexidine [38]; Triclosan [38]; Cetrimide, Aminoglycosides, Tetracycline [42]; Fluroquinolone [43]
CepA	-	Chlorhexidine [45,46]
oqxAB	-	Hydrogen peroxide [52]; Norfloxacin, Sodium dodecyl sulphate, Ethidium bromide, Acriflavine, Rhodamine G, Benzalkonium chloride [53]
qacE	*qacEΔ1*	Chlorhexidine [45]; Quaternary ammonium compounds [45]; Benzalkonium bromide [49]
SmvA	*ΔsmvA*	Chlorhexidine, Cetrimide, Cetyltrimethylammonium, Hexadecylpyridium chloride monohydrate [55]

after an exposure period of five minutes. The study also established that the percentage of viable *K. pneumoniae* after 2% and 1% glutaraldehyde, was greater when exposure to glutaraldehyde was performed after 15 days of desiccation of the biofilm.

Despite these, there is compelling evidence suggesting that sodium hypochlorite can clear and even inhibit biofilm formation in *K. pneumoniae* [62]. In the aforementioned, after establishing the MIC and MBC of 36 biofilm-forming *K. pneumoniae* being 1000μg/ml (0.1%) and 2000μg/ml (0.2%), respectively, the optical density (OD) was determined at 590nm [62]. Findings suggest that at 1000μg/ml, there was more than 30% inhibition reduction in biofilm formation for sensitive strains, as well as in the extended-spectrum beta-lactamase (ESBL)-producing and carbapenemase-producing isolates. Furthermore, the mentioned study demonstrated the biofilm clearance activity of sodium hypochlorite at 2000μg/ml and 5000μg/ml (0.5%) concentrations. Although the OD for all three groups (the sensitive, the ESBL-producing, and carbapenemase-producing isolates) increased after 24hr incubation at 2000μg/ml, the results were not statistically significant [62]. However, there was a statistically significant difference in the OD for all groups at 5000μg/ml. According to this study, the concentration of 0.5% sodium hypochlorite used for disinfection of benches and frequently contacted surfaces is adequate for the control of *K. pneumoniae*. These findings may be relevant when establishing disinfection strategies for carbapenem-resistant *K. pneumoniae* which has been found to show tolerance to sodium hypochlorite when compared with a control strain as demonstrated by Bhatia *et al.* [63].

Recently, there has been demonstration of reversing biocide resistance by harnessing the synergistic activity of Resveratrol and some biocides as reported by Migliaccio et al. [64], in bacteria including *K. pneumoniae*. Understanding biocide resistance is key in finding sustainable solutions to control biocide tolerant/resistant *K. pneumoniae*.

Discussion

In this review we analysed the available literature on BR in *K. pneumoniae*. With the COVID-19 pandemic there was a surge in the use of biocides as disinfectants and antiseptics in community and healthcare settings, at a global scale. Although little is known about the potential effect this had on BR, it is hypothesized that the increase in use of biocides may influence biocide-related selective pressure [65], thereby possibly changing the landscape of AMR and BR. Several studies, in fact, have demonstrated the phenomenon of BR (or tolerance) in *K. pneumoniae* and other ESKAPE pathogens. Some of the mechanisms with which this is achieved also influence antimicrobial drug susceptibility. This review has demonstrated that there are two main mechanisms of BR in *K. pneumoniae*: increased efflux of biocides via efflux pumps, and limited uptake of biocides due to biofilm formation. There are several putative genes and gene variations associated with this: *AcrAB-TolC*, *CepA*, *SmvA/SmvR*, *OqxAB* and *qacE/qacEΔ1*. Nevertheless, there are conflicting findings as to the extent that these genes and biofilm formation have in influencing antiseptic and/or AMR. This is a variation that can be also attributed to geographic and socio-economic aspects as they both affect the expression and phenotype thereof of the isolates [48].

The significance of BR is also minimal as concentrations used are high, and therefore high MICs do not necessarily equate to resistance. Furthermore, as conventional MIC experiments do not consider other environmental factors such as dust and debris, types of surfaces, humidity, exposure times and other variables [52], these values can only function as reference points to guide infection control. It should be noted that a harmonized terminology and methodological standards for BR testing have not yet been established, and this calls for an unambiguous classification of bacterial susceptibility to biocides to enable clear and comparable presentation of study results and the interpretation of available data [66].

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Conflict of interest statement

The authors declare no conflict of interest.

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Ethics statement

We do not need any ethical clearance/statement accompanying the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.infpip.2024.100360.

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