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Over-the-counter antibiotics compromising aminoglycoside activity

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Introduction: Antimicrobial resistance (AMR) is a global issue that needs addressing. While antibiotic stewardship has improved often by restricting antibiotic use, some antibiotics that are still sold legally over the counter (OTC), notably in sore throat medications. Recent findings suggest OTC antibiotics could trigger cross-resistance to antibiotics used in clinical treatments, whether systemic or topical. Here we investigated the impact of three antibiotics contained in OTC sore throat medicines on emerging AMR *in vitro*.

Methods: Bacterial pathogens were exposed to a bactericidal concentration of an aminoglycoside in the presence or absence of a during-use concentration of bacitracin, gramicidin or tyrothricin in a time–kill assay. Damage to the bacterial membrane was also investigated by measuring potassium leakage and membrane potential alteration post-OTC antibiotic exposure.

Results: Gramicidin (15 μg/mL) significantly decreased the bactericidal activity of amikacin, tobramycin or gentamicin in *Acinetobacter baumannii*. It also decreased gentamicin bactericidal activity in *Enterobacter cloacae*, *Escherichia coli* and *Klebsiella pneumoniae*, while tyrothricin decreased the aminoglycoside efficacy in *E. cloacae* and *E. coli*. Gramicidin significantly decreased bacterial membrane potential and caused significant potassium leakage.

Conclusion: Gramicidin and to some extent tyrothricin impacted aminoglycoside efficacy by affecting membrane potential, which is essential for aminoglycosides uptake. Thus, some OTC antibiotics can interfere with aminoglycoside activity, which could in turn affect treatment efficacy. Although the likelihood of OTC antibiotics and aminoglycosides being used at the same time might not be common, this research highlights one potential reason for OTC antibiotics' usage to result in treatment failure and their contribution to AMR development.

Introduction

Antimicrobial resistance (AMR) has become one of the world's major health issues and needs to be addressed urgently as, without intervention, the death rate could increase to 10 million deaths annually by 2050.[1] It is estimated that AMR is the cause of death of 33 000 people annually at a cost of €1.5 billion in Europe.[2] This is mainly attributed to both misuse and overuse of antibiotics.[3] To combat emerging AMR, a raft of measures should be implemented,[2] including better antibiotic stewardship.[2,4] Among bacterial pathogens, the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) are of particular concern having been identified as critical multidrug-resistant bacteria for which effective treatments are rapidly needed.[5] One area in which antibiotic stewardship needs to be improved is the use of antibiotic-containing medications to treat sore throats.[6] A sore throat is one of the most common ailments seen by community pharmacists and is often self-limiting.[7] Up to 80% of acute sore throats are of viral aetiology and therefore in most cases, antibiotic treatment is not needed to alleviate patients of their sore throat.[6] In other cases where antibiotics are required, this is recommended to be done via prescription rather than an OTC lozenge.

There are currently five antibiotics that have the WHO anatomical therapeutic classification (ATC) as antibiotics for use in OTC sore throat medications worldwide; bacitracin, fusafungine, gramicidin, neomycin and tyrothricin.[8] Fusafungine was banned in Europe in 2016 due to safety concerns, the risk of promoting antibiotic resistance and lack of evidence of therapeutic benefit.[9] The other four antibiotics are used for sore throat medications and also for other applications such as topical creams for skin infections or rectal ointments.[10]

Tyrothricin is a mixture of polypeptides isolated from the soil organism *Bacillus brevis* and consists of ~50%–70% tyrocidines and 25%–50% gramicidins.[11] The tyrocidines are basic cyclic peptides while the gramicidins are neutral linear peptides. Gramicidin is a polypeptide antibiotic that was discovered in the soil dwelling organism *Brevibacillus brevis*.[12] Gramicidin is also an AMP that is neutral and linear and can be classed as an ionophoric antibiotic.[13] Bacitracin was first isolated from *Bacillus subtilis* and is a polypeptide antibiotic that inhibits peptidoglycan synthesis.[14]

Whereas previous literature has claimed that resistance does not develop to these antibiotics,[15] recent literature demonstrated that exposure of organisms to these OTC antibiotics can cause the development of cross-resistance to clinical antibiotics such as ciprofloxacin, fusidic acid, beta-lactams and aminoglycosides.[16] An article by Short *et al*. demonstrated how benzalkonium chloride (BZK) can depolarize bacterial membranes thus effecting the uptake of aminoglycosides into bacterial cells and stop them from exerting their bactericidal effect.[17] This also led to the conclusion that this antibiotic antagonism could compromise aminoglycoside treatment and promote the evolution of resistance. We hypothesize that OTC antibiotics, particularly gramicidin and tyrothricin, could have the same effects. It is known that these antibiotics have a strong bactericidal activity against Gram-positive bacteria as a result of the formation of pores and the rapid depolarization of cell membranes.[11] These antibiotics have little to no efficacy in Gram-negative bacteria but could still interact with the cell.

This study aims to evaluate the impact of OTC antibiotics on the efficacy of topical or systemic aminoglycoside antibiotics in a range of bacteria and to investigate the mechanisms of potential antibiotic antagonism.

Methods and materials

Bacterial strains, culture conditions and antibiotic stock preparation

The bacterial strains used for testing were culture collection strains and included *Acinetobacter baumannii* Bouvet and Grimont ATCC 19568, *Enterobacter cloacae* ATCC 13047 (spinal fluid isolate), *Escherichia coli* ATCC 25922 (CLSI control strain for antimicrobial susceptibility testing) and *Klebsiella pneumoniae* ATCC 13883 (strain for antimicrobial preservative testing and bioresistance testing). These bacterial species are part of the ESKAPE pathogens that cause significant clinical challenges.[18]

Liquid bacterial cultures were grown at 37°C under shaking at 120 rpm overnight in cation-adjusted Müller–Hinton broth (MHB) for testing. For routine maintenance, bacteria were grown on tryptone soya agar (TSA) overnight at 37°C and stored at 4°C. Aminoglycosides (amikacin, gentamicin, tobramycin) used in this study were selected because they are the main agents in this drug class used as systemic treatment in clinical practice.[19] Aminoglycoside stock solutions (1 mg/mL) and bacitracin stock solution (250 IU/mL) in deionized sterile water were filtered sterilized using a 0.22 μm nitrocellulose filter. Gramicidin and tyrothricin stock solutions were dissolved in methanol at a concentration of 750 and 10 000 μg/mL, respectively. These stocks were not filter sterilized as this affects antibiotic efficacy, but sterility of the solutions was checked by plating an aliquot of the stocks on TSA plates. All antibiotic stocks were kept in the dark at 4°C for a maximum of 4 weeks.

Measurement of minimum inhibitory concentration (MIC)

Aminoglycoside MIC were determined using a standard microbroth-dilution test.[20] Aminoglycoside stock solutions were serially diluted 2-fold in sterile deionized water to give a final concentration range of 0.25–128 μg/mL. Bacterial test inocula were prepared in MHB to give a final concentration of 5×10^5 cfu/mL.

Table 1. Concentration of aminoglycosides used for each co-exposure assay. This corresponded to twice the MIC

Organism	Antibiotic	MIC (μg/mL)	Final concentration (μg/mL)
A. baumannii	Amikacin	1	2
	Gentamicin	1	2
	Tobramycin	1	2
E. cloacae	Gentamicin	2	4
E. coli	Gentamicin	2	4
K. pneumoniae	Gentamicin	1	2

Co-exposure time–kill assay

The co-exposure assay was based on the time to kill assay from Short *et al*.[17] Overnight liquid bacterial cultures (5 mL) were pelleted by centrifugation at 3000**g** for 10 minutes and resuspended in 5 mL of phosphate buffered saline (PBS). One hundred microlitres of washed culture was then added to 5 mL of MHB and incubated at 37°C, shaking at 120 rpm for 2 hours. After incubation, the aminoglycosides were added to the bacterial test suspension; the concentration used depended on the aminoglycoside and the bacterial species (Table 1).

One hundred microlitres of the OTC antibiotic stocks were also added to the test suspension to give the following final concentrations: gramicidin 15 μg/mL, tyrothricin 200 μg/mL and bacitracin 5 IU/mL. These during-use concentrations were determined by using the largest amount of OTC antibiotic in any one product (sore throat lozenge) within Europe and the documented average amount of saliva products that dissolved in it. When an antibiotic lozenge is used, instructions are to slowly dissolve it in the mouth, which should take ~30 minutes.[21] The average person produces between 0.5 and 1.5 L of saliva per day, therefore an approximate amount of saliva produced in 30 minutes is 20 mL.[22] Negative controls contained only aminoglycoside and positive control consisted of aminoglycosides co-exposed with BZK (4 μg/mL), a well-described membrane active antiseptic that has previously been shown to compromise aminoglycoside activity.[17] After the addition of antibiotics, bacterial concentration was immediately evaluated by taking 20 μL from the test suspension and drop counting on TSA using the Miles and Misra method following serial dilution in PBS. The test suspensions were then incubated at 37°C, under shaking at 120 rpm for 3 hours. After incubation, bacterial concentration was enumerated as described before. All TSA plates were incubated at 37°C overnight. Colonies in each drop were counted and the average was taken. The final colony forming units per millilitre was calculated and $\log_{10}$ reduction at 3 hours was compared with the controls.

Membrane potential assay

To measure the change in membrane potential, the BacLight™ bacterial membrane potential kit was used. Unlike the commonly used BacLight™ viability kit, which measures membrane integrity, the BacLight™ bacterial membrane potential kit uses a fluorescent dye DiOC2(3) (3,3′-diethyloxacarbocyanine iodide) to measure bacterial membrane potential. *A. baumannii* was used to measure the effects of OTC antibiotics on bacterial membrane potential due to technical difficulties of Enterobacteriaceae not interacting with the fluorescent dye. Five millilitres of overnight cultures of *A. baumannii* were pelleted by centrifugation

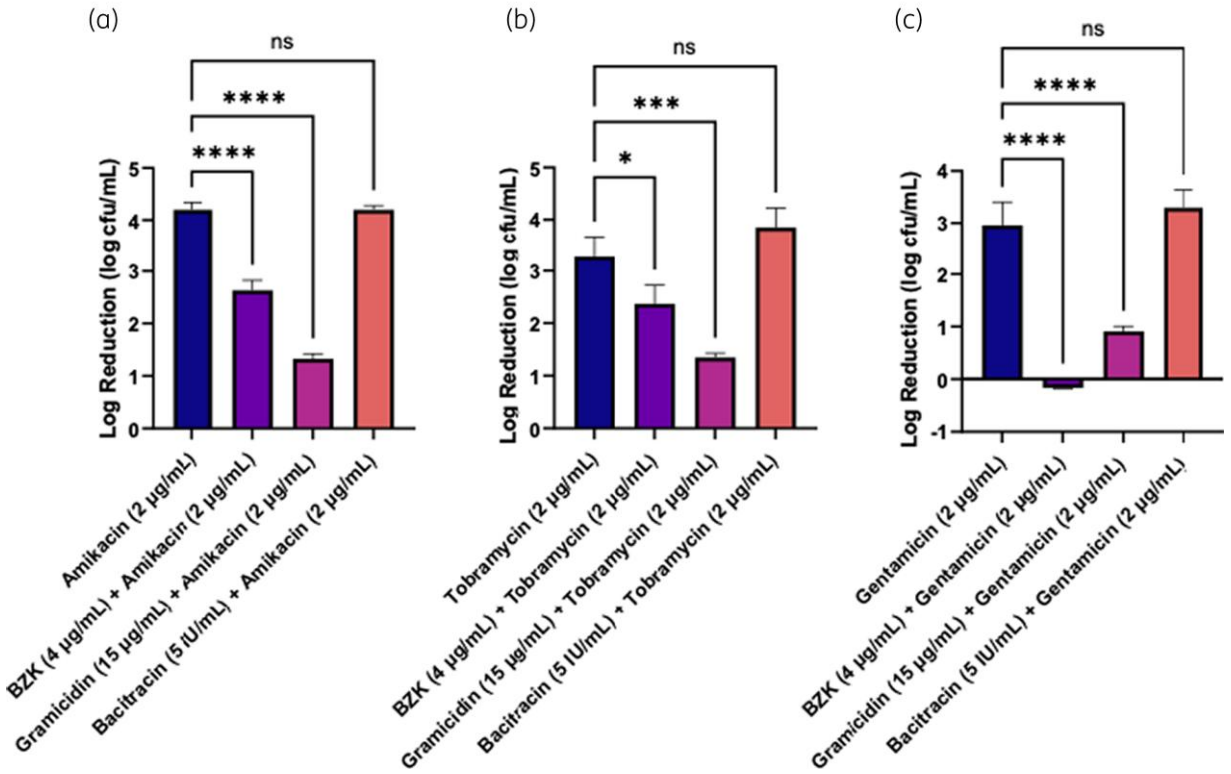

Figure 1. Bactericidal efficacy of aminoglycoside co-exposed OTC antibiotics in *A. baumannii*. (a) *A. baumannii* treated with amikacin (2 µg/mL) for 3 hours. There was a significant difference in $\log_{10}$ reduction when co-exposed with BZK (4 µg/mL; $P<0.0001$) or gramicidin (15 µg/mL; $P<0.0001$). When co-exposed to bacitracin (5 IU/mL) the difference in bactericidal efficacy to amikacin alone was not significant ($P>0.9999$). (b) *A. baumannii* treated with tobramycin (2 µg/mL) for 3 hours. Tobramycin efficacy was significantly reduced when co-exposed to either BZK (4 µg/mL; $P=0.0401$) or gramicidin (15 µg/mL; $P=0.0004$) but not with bacitracin (5 IU/mL; $P=0.2085$). (c) *A. baumannii* treated with gentamicin (2 µg/mL) for 3 hours. Gentamicin bactericidal efficacy was significantly decreased when exposed to BZK (4 µg/mL; $P<0.0001$) or gramicidin (15 µg/mL; $P<0.0001$) but not with bacitracin (5 IU/mL; $P=0.5136$). ns, not significant ($P>0.05$), $^{*}P\leq0.05$, $^{**}P\leq0.01$, $^{***}P\leq0.001$, $^{****}P\leq0.0001$ (raw data are available in Table S1a–c, available as Supplementary data at *JAC* Online). This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

at 3000**g** for 10 min and resuspended in 5 ml of MHB. The bacterial suspension was adjusted to a cell density of ~10^8 cfu/mL in MHB. One hundred microlitres of gramicidin (750 µg/mL) and bacitracin (250 IU/mL) stocks were added to 5 mL of adjusted cell suspension to give final concentrations of 15 µg/mL and 5 IU/mL, respectively. A negative control (polarized cells) consisting of no antibiotic treatment was used. A solvent control, cells treated with methanol, was also added. Suspensions were incubated for 3 hours at 37°C under shaking at 120 rpm. After incubation, cultures were diluted 100-fold in PBS to give an approximate cell density of 10^6 cfu/mL. One millilitre of suspension was aliquoted to a flow cytometry tube from the antibiotic exposed and untreated tubes. Two additional flow cytometry tubes were aliquoted from the untreated sample for the unstained and positive (depolarized) controls. To the positive control tube, 10 µL of 500 µM CCCP (carbonyl cyanide 3-chlorophenylhydrazone) was added and mixed for 10 seconds to give a final concentration of 5 µM CCCP. To each flow cytometry tube, except the unstained control, 10 µL of 3 mM DiOC2(3) was added and incubated for 30 min at room temperature before analysing by flow cytometry. Samples were analysed using a BD LSR Fortessa flow cytometer. The channels used for detecting the green and red fluorescence were the FITC-A and PE-Texas Red-A channels, respectively. The forward and side scatter of the flow cytometer was adjusted using the unstained cells to determine the gating for the bacterial population. In total, each sample had 10 000 events recorded. The mean red and green fluorescence was recorded and compared between samples to determine the change in membrane potential.

Potassium leakage

A. baumannii was grown overnight in 5 mL of MHB at 37°C, under shaking at 120 rpm. The culture was then washed three times in deionised sterile water by centrifuging at 3000**g**. One hundred microlitres of triple-washed culture were added to 5 mL of deionised sterile water. One hundred microlitres of gramicidin and bacitracin were then added to give final concentrations of 15 µg/mL and 5 IU/mL, respectively. Negative controls consisting of water or methanol and a positive control consisting of cells treated with 4 µg/mL BZK were used. Samples were incubated statically at room temperature for 3 hours. After incubation, the cultures were filtered into sterile tubes through a 0.22-µm nitrocellulose filter membrane. The potassium in each cell-free sample was measured using inductively coupled plasma spectrometry and concentrations were compared between samples to determine leaked potassium.

Statistical analysis

All experiments were conducted in biological triplicate. Statistical significance was determined by one-way ANOVA followed by Tukey's *post hoc* test on the data using GraphPad Prism (GraphPad Prism version 9.5.1. for

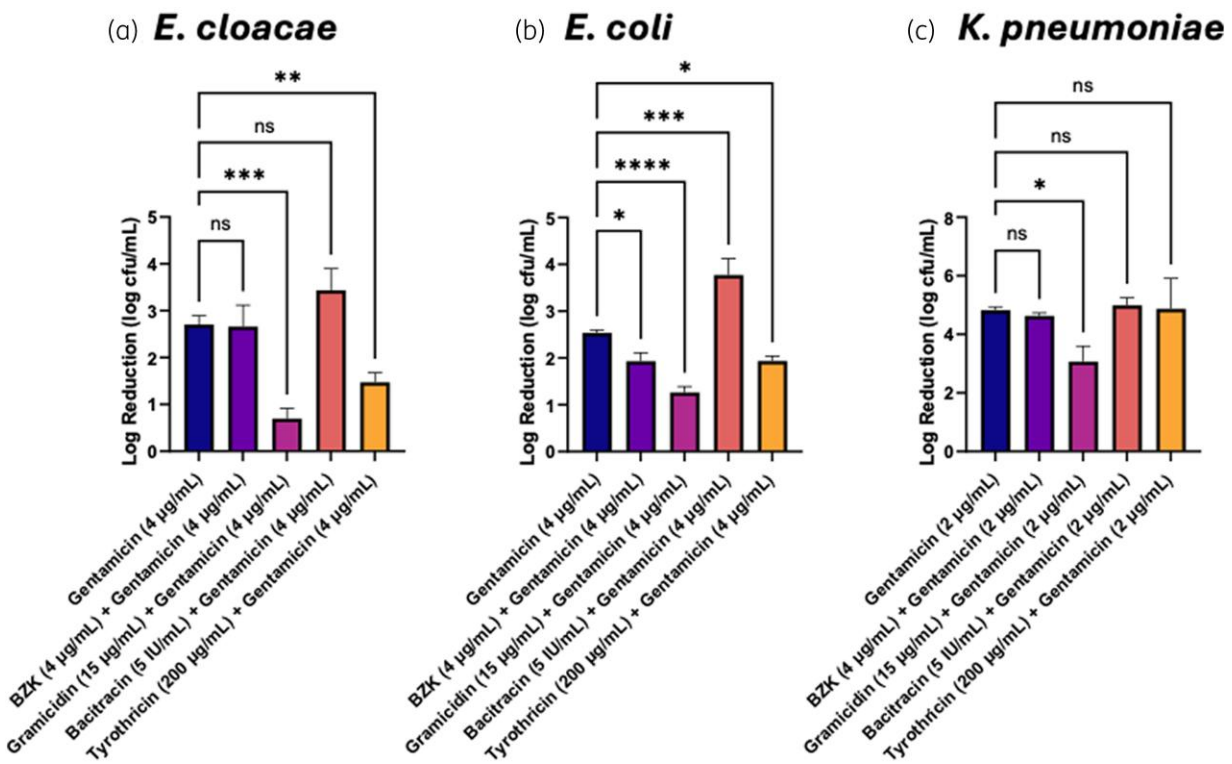

Figure 2. Bactericidal efficacy of aminoglycoside co-exposed OTC antibiotics in Enterobacteriaceae. (a) *E. cloacae* treated with gentamicin for 3 hours. Co-exposed to gramicidin or tyrothricin significantly decreased gentamicin efficacy when compared to gentamicin treatment alone ($P=0.0002$ and $P=0.0074$ respectively). (b) *E. coli* cultures treated with gentamicin for 3 hours. There was a significant decrease in gentamicin efficacy when cultures were co-exposed to BZK ($P=0.0217$), gramicidin ($P<0.0001$) or tyrothricin ($P=0.0001$). (c) *K. pneumoniae* treated with gentamicin for 3 hours. Co-exposure to gramicidin significantly decreased efficacy when compared to gentamicin alone ($P=0.0172$). ns, not significant ($P>0.05$), $^{*}P\leq0.05$, $^{**}P\leq0.01$, $^{***}P\leq0.001$, $^{****}P\leq0.0001$ (raw data available in Table S2a–c). This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Windows).[23] Time–kill experimental data were log-transformed before analysis.

Results

Aminoglycoside MICs for the different test bacteria are reported in Table 1.

Gramicidin and BZK protect A. baumannii *against aminoglycoside*

Amikacin (2 μg/mL) was strongly bactericidal (4.20 ± 0.14 $\log_{10}$ reduction in cfu/mL) against *A. baumannii* after 3 hours exposure (Figure 1a), but when co-exposed with either BZK (4 μg/mL) or gramicidin (15 μg/mL), its bactericidal activity significantly ($P<0.0001$) decreased to 2.63 ± 0.19 $\log_{10}$ cfu/mL with BZK and 1.33 ± 0.09 $\log_{10}$ cfu/mL with gramicidin (Figure 1a). Similar results were observed with tobramycin (3.27 ± 0.40 $\log_{10}$ reduction alone; Figure 1b) and gentamicin (2.96 ± 0.44 $\log_{10}$ reduction alone; Figure 1c), with a significant reduction in their bactericidal efficacy when exposed to BZK (2.37 ± 0.35 $\log_{10}$ reduction ($P=0.401$) for tobramycin (Figure 1b); -0.17 ± 0.01 $\log_{10}$ reduction ($P<0.0001$) for gentamicin (Figure 1c), or gramicidin (1.35 ± 0.08 $\log_{10}$ reduction ($P=0.0004$) for tobramycin (Figure 1b); 0.91 ± 0.09 $\log_{10}$ reduction ($P<0.0001$) for gentamicin (Figure 1c). By contrast, bacitracin (5 IU/mL) did not reduce the bactericidal efficacy of any of the aminoglycosides (Figure 1).

OTC antibiotics and BKC can protect some ESKAPE Enterobacteriaceae against gentamicin

Gentamicin at the concentrations tested (Table 1) was confirmed to be bactericidal after 3 hours exposure against *E. cloacae* (2.71 ± 0.19 $\log_{10}$ reduction), *E. coli* (2.53 ± 0.06 $\log_{10}$ reduction) and *K. pneumoniae* (4.82 ± 0.11 $\log_{10}$ reduction) (Figure 2).

BZK (4 μg/mL) did not affect the bactericidal efficacy of gentamicin (2.67 ± 0.46 $\log_{10}$ reduction; $P=0.9998$) in *E. cloacae* (Figure 2a). Gentamicin co-exposure to gramicidin (15 μg/mL) or tyrothricin (200 μg/mL) significantly reduced the efficacy of the aminoglycoside (0.70 ± 0.22 $\log_{10}$ reduction with gramicidin and 1.47 ± 0.20 $\log_{10}$ reduction with tyrothricin; Figure 2a).

In *E. coli*, co-exposure to BZK (4 μg/mL), gramicidin (15 μg/mL) or tyrothricin (200 μg/mL) negatively affected the efficacy of gentamicin (1.93 ± 0.18 $\log_{10}$ reduction ($P=0.0271$) with BZC; 1.26 ± 0.13 $\log_{10}$ reduction ($P<0.0001$) with gramicidin; 1.47 ± 0.20 $\log_{10}$ reduction ($P=0.225$; Figure 2b). By contrast, the combination of gentamicin with bacitracin significantly contributed to an increased bactericidal efficacy of the aminoglycoside (3.77 ± 0.35 $\log_{10}$ reduction; $P=0.0001$) (Figure 2b).

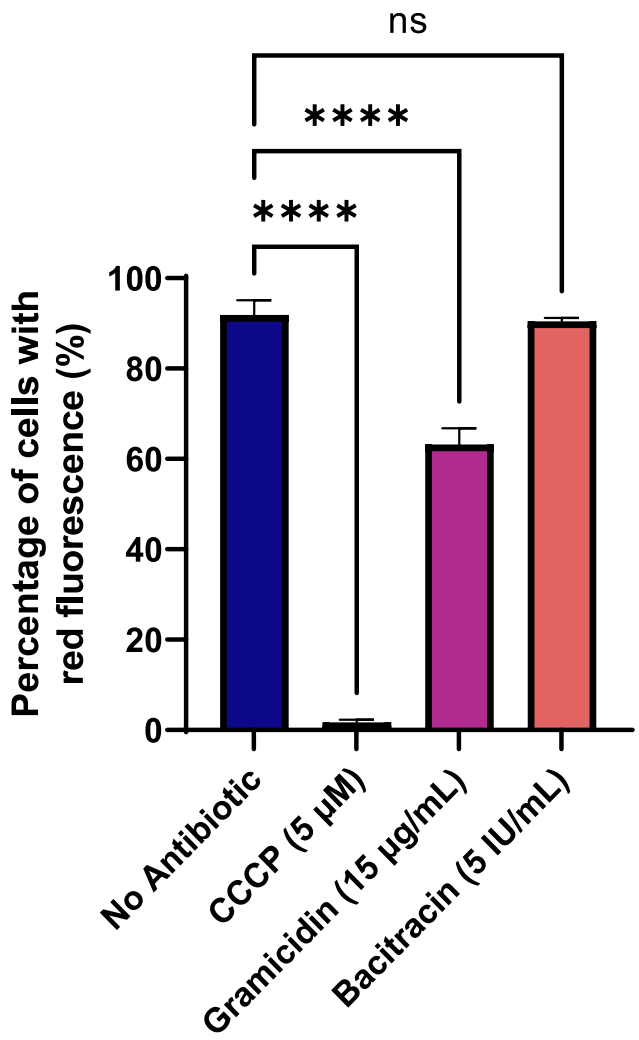

Figure 3. *A. baumannii* membrane potential following exposure to OTC antibiotics. CCCP (5 μM) was used as a positive control. ns, not significant ($P > 0.05$), *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, **** $P \leq 0.0001$. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

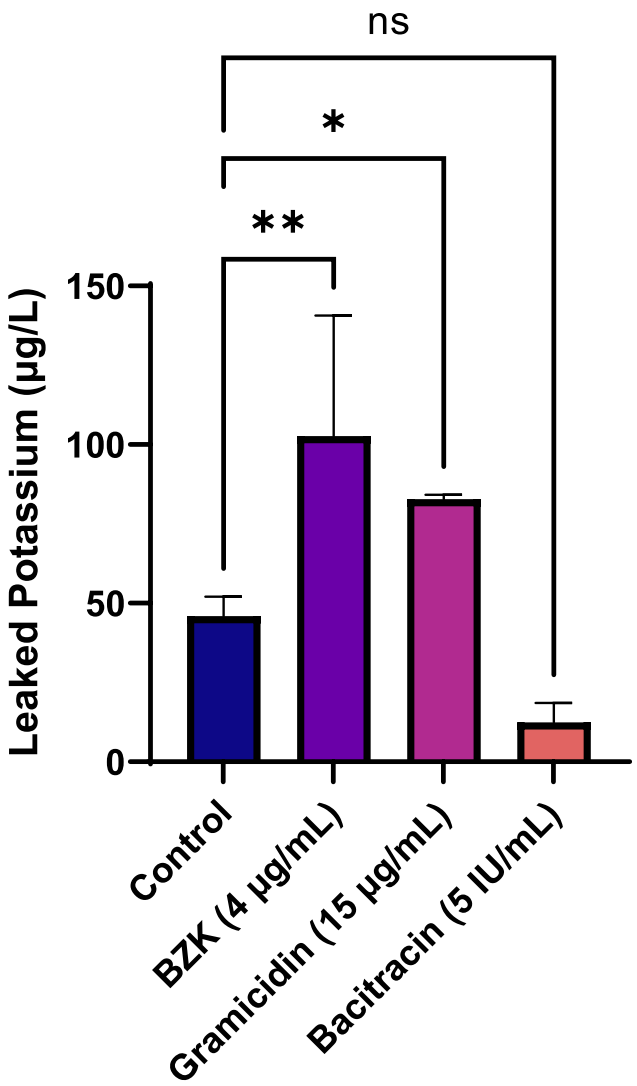

Figure 4. Potassium concentration in solution following exposure to OTC antibiotics in *A. baumannii*. ns, not significant ($P > 0.05$), *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

In *K. pneumoniae*, co-exposure data were different (Figure 2c). Only the combination of gentamicin (2 μg/mL) with gramicidin (15 μg/mL) significantly decreased the efficacy of the aminoglycoside (3.07 ± 0.53 $\log_{10}$ reduction; $P = 0.0172$) (Figure 2c). Co-exposure of gentamicin with BZK (4 μg/mL, bacitracin (5 IU/mL) or tyrothricin (200 μg/mL) did not result in statistically significant changes in bactericidal activity ($P = 0.9913$, $P = 0.9947$, $P = 0.9947$, respectively).

Gramicidin and membrane potential

There was a change in cell membrane potential in *A. baumannii* suspensions exposed to gramicidin (15 μg/mL) but not the cultures exposed to bacitracin (5 IU/mL) after 3 hours of exposure. When bacteria were not exposed to an antibiotic, their cell membrane potential remained intact with an average of $91.90\% \pm 3.20\%$ of cells exhibiting red fluorescence (Figure 3). After exposure to CCCP (5 μM) for 10 seconds, *A. baumannii* cell membrane potential was supressed with treated cells having an average red fluorescence of $1.66\% \pm 0.65\%$ ($P < 0.0001$). Cell membrane potential was also lowered in cultures exposed to gramicidin (15 μg/mL) with the average red fluorescence being $63.20\% \pm 3.62\%$ ($P < 0.0001$). By contrast, cells' exposure to bacitracin (5 IU/mL) did not significantly decrease the mean red fluorescence ($90.47\% \pm 0.70\%$; $P = 0.8890$).

Gramicidin and BZK cause potassium to leak from bacteria

When bacteria were not exposed to antibiotics, potassium concentration in solution was 45.95 ± 6.16 μg/L (Figure 4). Exposure of *A. baumannii* to BZK (control) and gramicidin (15 μg/mL) led to a significant leakage in potassium (102.70 ± 38.02 μg/L ($P = 0.0074$) with BZK and 82.83 ± 1.30 μg/L ($P = 0.0493$) with

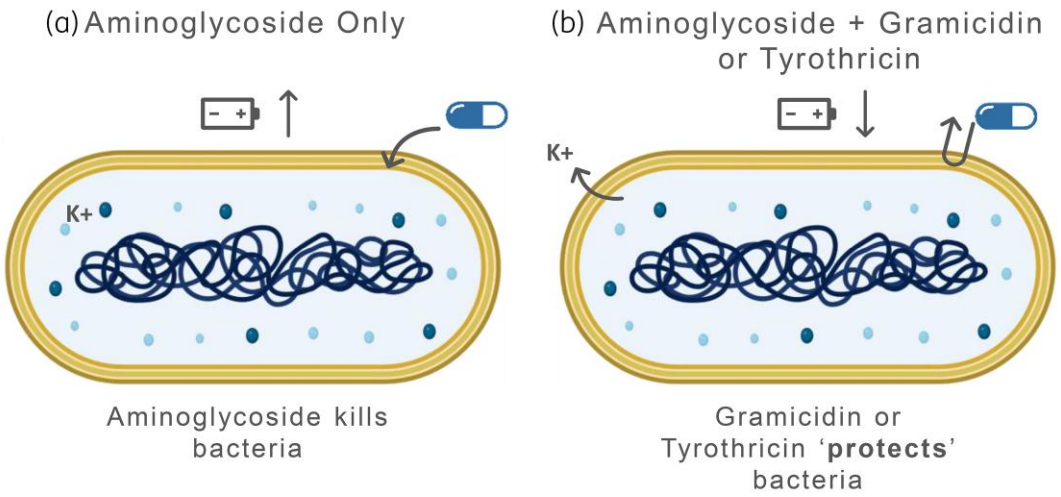

Figure 5. Mechanism of protection during co-exposure with gramicidin or tyrothricin. (a) When bacteria are exposed to only aminoglycosides, the potassium homeostasis in maintained and the cells remain polarized. This allows aminoglycosides to enter bacteria and kill them. (b) When co-exposed with either gramicidin or tyrothricin, the OTC antibiotics create pores in the bacterial membranes. This cause intracellular potassium to leak out and therefore depolarizes cells. Aminoglycosides cannot enter depolarized cells and therefore the gramicidin or tyrothricin will 'protect' the bacteria from aminoglycoside activity. The battery represents the bacterial membrane potential and the pill capsule represents aminoglycoside treatments. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

gramicidin. Bacitracin (5 IU/mL) did not lead to the leakage of potassium in solution (12.43 ± 6.09 μg/L; $P = 0.0684$).

Discussion

Aminoglycosides inhibit protein synthesis by binding to the 30S ribosome and therefore need to enter bacterial cells to be effective.[24] Here we explored the bactericidal activity of amikacin, gentamicin, tobramycin following co-exposure to some polypeptide OTC antibiotics and the antiseptic BZC. Another OTC antibiotic, neomycin,[8] which is also an aminoglycoside, was not studied in the co-exposure effect, as it would be unlikely to affect the bacterial membrane. Also, neomycin (250 μg/mL) was found to inhibit the growth of *E. cloacae* (data not shown).

Aminoglycoside activity is dependent on the bacterial cell membrane potential, as this potential is required for the aminoglycosides to enter into cells.[25] The cell membrane potential is often determined by the concentration of intracellular ions, in particular potassium ions.[26] Our results showed that in bacterial cells that have only been treated with the aminoglycosides, potassium homeostasis is maintained (Figure 4) and therefore the cell membrane potential remains polarized (Figure 3), allowing aminoglycosides to enter cells and exert their bactericidal effect (Figure 5a). However, cells co-exposed with gramicidin led to potassium leakage (Figure 4) and a decrease in red fluorescence. This indicates that the DiOC2(3) associates with cells less after gramicidin treatment as the bacterial membranes are being depolarized (Figure 3). This prevents aminoglycosides to enter bacterial cells and subsequently decreases the aminoglycoside bactericidal efficacy (Figure 1), demonstrating a 'protection' effect (Figure 5b). Gramicidin acts as an ionophore and therefore has similar effects to compounds such as indole.[27] Gramicidin forms channels across the cell membrane and disrupts the ionic homeostasis resulting in membrane depolarization and leakage of monovalent cations.[28]

The explanation for the decreased aminoglycoside efficacy when co-exposed to tyrothricin (Figures 1 and 2) is likely to be similar to gramicidin. Tyrothricin is partly made from gramicidin so has a similar mechanism of action, but it also contains another antimicrobial peptide, tyrocidine. This mechanism of action is currently disputed but is thought to work by binding of the bacterial cell membrane and embedding within it forming pores.[29,30]

By contrast, bacitracin's mechanism of action is to inhibit the formation of the bacterial cell wall. This is done by the bacitracin forming a complex with part of the bacterial cell wall, C55-isoprenyl pyrophosphate[14] with no impact on cell membrane potential. This would explain co-exposure to bacitracin has no impact on aminoglycoside efficacy (Figures 1 and 2).

The use of OTC antibiotics has been questioned for many years due to safety concerns and lack of therapeutic benefit, with some countries banning their use.[6] Our study indicates that OTC antibiotics such as gramicidin and tyrothricin have the potential to antagonize aminoglycoside activity. Aminoglycosides are crucial treatments used by clinicians for sometimes life-threatening infections.[31] At present, it remains to be shown that OTC antibiotics and aminoglycosides can be present at the same time against bacterial pathogens in a patient. Aminoglycosides are poorly absorbed orally and are therefore often administered by injection.[32] However, there are instances where aminoglycosides are given orally such as neomycin in the treatment of hepatic coma or preoperative sterilization or paromomycin in the treatment of parasitic infections. Aminoglycosides can also be given by inhalation, such as the use of tobramycin in cystic fibrosis patients or liposomal amikacin in the treatment of *Mycobacterium avium* complex.[32] In both these examples, these aminoglycosides could potentially come into contact with OTC antibiotics. In addition,

aminoglycosides are also used to treat external infections such as eyes, wounds or ears. While it is unlikely that these antibiotics will come into contact with aminoglycosides when contained in sore throat medications, OTC antibiotics are used in other types of medication such as topical skin preparations.[10]

It has been suggested that OTC antibiotics do not directly cause the development of AMR[11] because they are antimicrobial peptides, without a defined target site and strong bactericidal action. Bacitracin has been documented to be effective against Gram-positive bacteria only,[33] while gramicidin is active mostly against Gram-positive but also against some Gram-negative organisms,[34] although reported gramicidin MIC for the test bacteria vary between studies.[16,34]

A more recent study showed that these OTC antibiotics can elicit responses that generate not resistance to the OTC antibiotics themselves but cross-resistance to clinical antibiotics.[16] Here, we showed that gramicidin and tyrothricin can decrease significantly the efficacy of aminoglycosides in Gram-negative bacteria and provided an explanation as to the mechanism of antibiotic antagonism.

Conclusion

This study highlights how the exposure of gramicidin and tyrothricin can interfere with the bactericidal activity of aminoglycosides. This is due to the disturbance of the bacterial membrane potential that is required for the uptake of aminoglycosides into bacteria. It therefore needs to be considered whether other membrane active agents, such as biocides or surfactants, could also 'protect' bacteria from aminoglycoside activity. This study raises some concerns regarding the use of some OTC antibiotics in products for sore throats and AMR, and suggests that a review of the ATC classification for these products by the WHIO may be warranted.

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Transparency declarations

G. Coutinho, A. Shephard and T. Pillay are employee of Reckitt. A. Shephard is also a Shareholder. All other authors: none to declare.

Supplementary data

Tables S1 and S2 are available as Supplementary data at *JAC* Online.

References

1 Howard SJ, Catchpole M, Watson J *et al*. Antibiotic resistance: global response needed. *Lancet Infect Dis* 2013; **13**: 1001–3. https://doi.org/10.1016/S1473-3099(13)70195-6

2 O'Neill J. The review on antimicrobial resistance, tackling drug-resistant infections globally: Final report and recommendations. 2016. https://amr-review.org

3 Machowska A, Lundborg CS. Drivers of irrational use of antibiotics in Europe. *Int J Environ Res Public Health* 2018; **16**: 1–14. https://doi.org/10.3390/ijerph16010027

4 Laxinarayan R, Duse A, Wattal C *et al*. Antibiotic resistance—the need for global solutions. *Lancet Infect Dis* 2013; **13**: 1057–98. https://doi.org/10.1016/S1473-3099(13)70318-9

5 Miller WR, Arias CA. ESKAPE pathogens: antimicrobial resistance, epidemiology, clinical impact and therapeutics. *Nat Rev Microbiol* 2024; **22**: 598–616. https://doi.org/10.1038/s41579-024-01054-w

6 Essack S, Bell J, Burgoyne DS *et al*. Topical (local) antibiotics for respiratory infections with sore throat: an antibiotic stewardship perspective. *J Clin Pharm Ther* 2019; **44**: 829–37. https://doi.org/10.1111/jcpt.13012

7 Mantzourani E, Wasag D, Cannings-John R *et al*. Characteristics of the sore throat test and treat service in community pharmacies (STREP) in Wales: cross-sectional analysis of 11304 consultations using anonymized electronic pharmacy records. *J Antimicrob Chemother* 2023; **78**: 84–92. https://doi.org/10.1093/jac/dkac358

8 World Health Organization. R02AB—antibiotics. 2023. https://www.whocc.no/atc_ddd_index/?code=R02AB

9 European Medicines Agency. CMDh endorses revocation of authorisations for fusafungine sprays used to treat airway infections. 2016. https://www.ema.europa.eu/en/documents/referral/fusafungine-article-31-referral-cmdh-endorses-revocation-authorisations-fusafungine-sprays-used_en.pdf

10 Both L, Botgros R, Cavaleri M. Analysis of licensed over-the-counter (OTC) antibiotics in the European Union and Norway, 2012. *Euro Surveill* 2015; **20**: 1–8. https://doi.org/10.2807/1560-7917.ES.2015.20.34.30002

11 Lang C, Staiger C. Tyrothricin—an underrated agent for the treatment of bacterial skin infections and superficial wounds. *Pharmazie* 2016; **71**: 299–305. https://doi.org/10.1691/ph.2016.6038

12 Dubos RJ. Studies on a bactericidal agent extracted from a soil *Bacillus*. *J Exp Med* 1939; **70**: 1–10. https://doi.org/10.1084/jem.70.1.1

13 Busath D, Szabo G. Gramicidin forms multi-state rectifying channels. *Nature* 1981; **294**: 371–3. https://doi.org/10.1038/294371a0

14 Stone KJ, Strominger JL. Mechanism of action of bacitracin: complexation with metal ion and C55-isoprenyl pyrophosphate. *Proc Natl Acad Sci USA* 1971; **68**: 3223–7. https://doi.org/10.1073/pnas.68.12.3223

15 Stauss-Grabo M, Atiye S, Le T *et al*. Decade-long use of the antimicrobial peptide combination tyrothricin does not pose a major risk of acquired resistance with Gram-positive bacteria and *Candida* spp. *Pharmazie* 2014; **69**: 838–41. https://doi.org/10.1691/ph.2014.4686

16 Wesgate R, Evangelista C, Atkinson R *et al*. Understanding the risk of emerging bacterial resistance to over the counter antibiotics in topical sore throat medicines. *J Appl Microbiol* 2020; **129**: 916–25. https://doi.org/10.1111/jam.14682

17 Short FL, Lee V, Mamun R *et al*. Benzalkonium chloride antagonizes aminoglycoside antibiotics and promotes evolution of resistance. *EBioMedicine* 2021; **73**: 103653. https://doi.org/10.1016/j.ebiom.2021.103653

18 Mulani MS, Kamble EE, Kumkar SN *et al*. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. *Front Microbiol* 2019; **10**: 539. https://doi.org/10.3389/fmicb.2019.00539

19 European Committee on Antimicrobial Susceptibility Testing (EUCAST). Disk diffusion method for antimicrobial susceptibility testing; version 7.0. 2019. www.eucast.org/ast_of_bacteria/disk_diffusion_methodology/

20 International Organisation for Standardisation (ISO). Clinical laboratory testing and in vitro diagnostic test systems—susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices—part 1: reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases. 2019. ISO 20776-1:2019.

21 Maheshwari R, Jain V, Ansari R *et al*. A review on lozenges. *Br Med Bull* 2013; **1**: 35–43.

22 Iorgulescu G. Saliva between normal and pathological. Important factors in determining systemic and oral health. *J Med Life* 2009; **2**: 303–7.

23 GraphPad Prism version 9.5.1 for Windows, GraphPad software, San Diego, CA, www.graphpad.com

24 Krause KM, Serio AW, Kane TR *et al*. Aminoglycosides: an overview. *Cold Spring Harb Perspect Med* 2016; **6**: a027029. https://doi.org/10.1101/cshperspect.a027029

25 Taber HW, Mueller JP, Miller PF *et al*. Bacterial uptake of aminoglycoside antibiotics. *Microbiol Rev* 1987; **51**: 439–57. https://doi.org/10.1128/mr.51.4.439-457.1987

26 Stautz J, Hellmich Y, Fuss MF *et al*. Molecular mechanisms for bacterial potassium homeostatis. *J Mol Biol* 2021; **433**: 166968. https://doi.org/10.1016/j.jmb.2021.166968

27 Chimerel C, Field CM, Piñero-Fernandez S *et al*. Indole prevents *Escherichia coli* cell division by modulating cell membrane potential. *Biochim Biophys Acta* 2012; **1818**: 1590–4. https://doi.org/10.1016/j.bbamem.2012.02.022

28 Meikle TG, Conn CE, Separovic F *et al*. Exploring the structural relationship between encapsulated antimicrobial peptides and the bilayer membrane mimetic lipidic cubic phase: studies with gramicidin A. *RSC Adv* 2016; **6**: 68685–94. https://doi.org/10.1039/C6RA13658C

29 Marques MA, Citron DM, Wang CC. Development of tyrocidine A analogues with improved antibacterial activity. *Bioorg Med Chem* 2007; **15**: 6667–77. https://doi.org/10.1016/j.bmc.2007.08.007

30 Pálffy R, Gardlik R, Behuliak M *et al*. On the physiology and pathophysiology of antimicrobial peptides. *Mol Med* 2009; **15**: 51–9. https://doi.org/10.2119/molmed.2008.00087

31 Serio AW, Keepers T, Andrews L *et al*. Aminoglycoside revival: review of a historically important class of antimicrobials undergoing rejuvenation. *EcoSal Plus* 2018; **8**: 0002. https://doi.org/10.1128/ecosalplus.esp-0002-2018

32 Joint Formulary Committee. *British National Formulary (Online)*. BMJ Group and Pharmaceutical Press, 2024.

33 Heal CF, Banks JL, Lepper PD *et al*. Topical antibiotics for preventing surgical site infection in wounds healing by primary intention. *Cochrane Database Syst Rev* 2016; **11**: CD011426. https://doi.org/10.1002/14651858.CD011426.pub2

34 Kondejewski LH, Farmer SW, Wishart DS *et al*. Modulation of structure and antibacterial and hemolytic activity by ring size in cyclic gramicidin S analogs. *J Biol Chem* 1996; **271**: 25261–8. https://doi.org/10.1074/jbc.271.41.25261