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ORIGINAL ARTICLE

The effect of nickel ions on the susceptibility of bacteria to ciprofloxacin and ampicillin

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Abstract

To explore the interaction effects of ciprofloxacin and ampicillin with nickel on the growth of bacteria, *Staphylococcus aureus* strain ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922 were used. Minimum inhibitory concentrations (MICs) were determined for nickel, ciprofloxacin and ampicillin, and the checkerboard method was used to assess their cumulative effects on bacterial growth. The interactions between the metal and antibiotics were assessed by the fractional inhibitory concentration (FIC). The MICs for ciprofloxacin and ampicillin were 0.31 and 1 mg/L for *E. faecalis*, 0.62 and 1 mg/L for *S. aureus* and 0.005 and 2.5 for *E. coli*, respectively. The MIC for nickel was 1000 mg/L for all bacteria. The FIC results for ciprofloxacin and nickel demonstrated an antagonistic effect of the two agents on the growth of *E. coli* and *E. faecalis* and an additive effect on *S. aureus*. The FICs for ampicillin and nickel demonstrated a synergistic effect on the growth of *E. faecalis* and *E. coli*. Different interactions of metals and antibiotics were observed depending on the bacteria and the type of antibiotic.

Keywords Nickel · Ciprofloxacin · Ampicillin · Bacteria

Introduction

Heavy metals are micronutrients, although at higher concentrations, they can have a toxic effect on living beings. Their importance is emphasised because of their non-biodegradability and accumulation in organisms (Atieh et al. 2017). Nickel (Ni) is a silvery-white heavy metal found in the Earth's core and present in living organisms, mostly in plants. It is of interest in medicine because it is the most common contact allergen in the world (Thyssen et al. 2007). Human nickel intake is mostly in food, with some foods such as chocolate, oatmeal and nuts being especially nickel rich, which can increase nickel intake to 900 µg/day (Flyvholm et al. 1984). Furthermore, it can be released into the human body by the oral corrosion of nickel-containing dental alloys used for dental instruments, restorations and orthodontic appliances (Wendl et al. 2017). There is some concerning evidence of elevated nickel levels in the tissues of patients who have had an orthodontic appliance for 1 year (Jamshidi et al. 2018).

Human organisms contain more prokaryotic bacterial cells than eukaryotic cells, referred to as human commensal bacteria or the human microbiota. The role of the microbiota is increasingly recognised as vital for health and homeostasis, with some terming it a human organ (Wang et al. 2007; O'Hara and Shanahan 2006). It is of major importance for modelling metabolic phenotypes, immune system function, nutrient absorption and protection against pathogens (Wang and Li 2015; Ley et al. 2006).

Commensals can exist in silent mode, but when introduced to a susceptible body location or to an immunocompromised, injured or diseased individual, they can cause

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serious morbidity. Their silent and asymptomatic introduction to the community presents an epidemiologic challenge (Nicolas-Chanoine et al. 2008). Some bacteria that demonstrate such behaviour are *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus faecalis* (Price et al. 2017). Heavy metals have also demonstrated the ability to alter the nature of bacteria (Dickinson et al. 2019). *S. aureus* is a gram-positive facultative anaerobe present as a commensal on human skin and, in 30% of the population, on the nasal mucosa (Mulcahy and McLoughlin 2016). The presence of *S. aureus* in the oral cavity is underestimated as well (Smith et al. 2003). It is also a bacterium responsible for invasive, life-threatening infections, and unfortunately, its strains demonstrate increased antimicrobial resistance (Dumitrescu et al. 2010). *E. coli* is a gram-negative facultative anaerobic bacterium that colonises all mammals (Finegold et al. 1983). It is primarily a colon inhabitant, although it can be found in the upper digestive tract and even in the oral cavity (Foster 2004; Zawadzki et al. 2017). Pathogenic strains are the leading cause of urinal tract infections (Guglietta 2017). *E. faecalis* is a gram-positive commensal of the digestive system. Like the previously mentioned bacteria, it can have a pathogenic effect, causing infections of the urinary tract, bacteraemia and endocarditis (Huycke et al. 1998).

Ciprofloxacin is a second-generation fluoroquinolone antibiotic with a wide activity spectrum (Oliphant and Green 2002). It was introduced into the market in 1987 (Torok et al. 2009). Initially, it was very effective even against methicillin-resistant *S. aureus* (Righter 1987), and although *S. aureus* strains are currently more resistant to it, it is still the drug of choice (Suleiman et al. 2012). Ciprofloxacin in combination with other antimicrobials can be effective against *E. faecalis*, although it is not the first antibiotic of choice (Holmberg et al. 2012). Moreover, ciprofloxacin affects commensal enterococci (de Lastours et al. 2017). Ampicillin is a broad-spectrum penicillin antibiotic used to treat gram-positive and gram-negative bacteria. It is effective against *S. aureus* but not against MRSA (Foxley et al. 2016). *E. coli* is often resistant to ampicillin (Vranic and Uzunovic 2016), while infections caused by some resistant strains of *E. faecalis* can be successfully treated with ampicillin (Conceição et al. 2014).

The interactions among the described bacteria, nickel and antibiotics are multifold. Although bacteria may benefit from lower concentrations of nickel (Kaluarachchi et al. 2010), higher concentrations have been shown to inhibit their growth (Macomber and Hausinger 2011). For the last 20–25 years, an interesting phenomenon has been described and intensively studied. Various strains of bacteria grown in the presence of subinhibitory concentrations of heavy metals, such as mercury, cobalt, copper, zinc and nickel, demonstrated resistance to these same metals. More significantly, bacteria that adapted to heavy metals demonstrated higher resistance to antibiotics (Resende et al. 2012; Calomiris et al. 1984; Nyamboya et al. 2013). The occurrence of strain resistance to antibiotics is one of the greatest challenges in modern medicine (Zaman et al. 2017). Moreover, metals can modulate an antibiotic effect. Both synergistic and antagonistic relationships between metals and antibiotics have been observed (Wolska et al. 2012; Freitas et al. 2018).

The aim of this study was to assess the concentrations of nickel ions that have a bacteriostatic effect on commensal bacteria. Furthermore, the potential interactions of nickel, antibiotics and commensal bacteria were tested.

Materials and methods

Strains and growth media

Staphylococcus aureus strain ATCC 29213 (American Type Cell Culture), *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 were used. The bacteria were kept frozen at − 80 °C with the addition of 10% glycerol. For each experiment, an aliquot was thawed and grown in Mueller–Hinton (MH) broth (Biolife Italiana, Milano, Italy) for 24 h at 37 °C and then subcultured on Mueller–Hinton (MH) agar (Biolife Italiana, Milano, Italy). The concentration of bacteria was determined by measuring the optical density (OD) at 600 nm. An OD of 1 at 600 nm corresponded to approximately 1×10^9 CFU/mL (colony forming units per mL). Three series of tenfold dilutions were made to obtain a bacterial suspension of 10^6 CFU/mL. Bacterial inocula were later verified by diluting, plating the dilutions onto MH agar, and incubating for 24 h at 37 °C.

Preparation of reagents

All reagents used were of analytical or molecular biology grade and purchased from Sigma-Aldrich. Stock solution of $NiCl_2 \times 6H_2O$ (Sigma-Aldrich, St. Louis, USA) was prepared in double-distilled water, adjusted to pH 7, passed through a 0.45-µm syringe filter and stored at 4 °C. The stock concentration was 8000 mg/L. Stock solutions of ciprofloxacin and ampicillin (Sigma-Aldrich, St. Louis, USA) were prepared in sterile saline solution (0.9% NaCl) and passed through a 0.45-µm syringe filter. The working concentrations of the $NiCl_2 \times 6H_2O$ solution were prepared by diluting the stock solution to reach concentrations of 2000–125 mg/L.

Determination of minimum inhibitory concentration (MIC)

A resazurin-based microdilution method was used to determine the minimum inhibitory concentrations (MICs) of nickel and ciprofloxacin, which were used in the checkerboard method described below. Serial twofold dilutions of reagents ranging from 2000 to 125 mg/L for nickel, 0.08 to 5 mg/L for ciprofloxacin and 0.12 to 4 mg/L for ampicillin were made in a microtiter plate (Vacutest Kima s.r.l., Italy). Bacterial suspension (1×10^6 CFU/mL per well) and resazurin (0.015% solution) (Sigma-Aldrich, St. Louis, USA) were added to each well. Three wells were used as sterility controls, and three were used as growth controls. After 24 h of incubation at 37 °C, the plates were read visually. The lowest concentration that did not show a change in colour (blue) was defined as the MIC. All dilutions were inoculated on MH agar and incubated for 24 h at 37 °C.

Checkerboard synergy method

To examine the interactions of metals and antibiotics and their effects on bacterial growth, a checkerboard method was used. Stock solutions and serial twofold dilutions of nickel and ciprofloxacin to at least twofold MIC were prepared in MH broth and distributed into the wells of a sterile microtiter plate. The metal of the combination was serially diluted along the ordinate, while the antibiotic was diluted along the abscissa. A bacterial inoculum of 1×10^6 CFU/mL in MH broth was prepared from each bacterial species and added, with resazurin (0.015% solution), to the wells with diluted reagents to reach a final volume of 200 µL. With this preparation process, different combinations of antibiotic and metal concentrations were obtained, and the concentration of metals diminished by rows while that of antibiotics diminished by columns. Columns 1 and 12 and rows A and H were filled with distilled water to prevent the inner wells from drying out. Column 2 was filled with nickel solution and served as a control and MIC confirmation. Row B, analogously, was a ciprofloxacin or ampicillin solution control. Rows C–G and columns 3–11 were filled with various combinations of nickel and ciprofloxacin or ampicillin. The plates were incubated at 37 °C for 24 h under aerobic conditions. Then, the growth of bacteria in the wells was read visually by the change in the colour of resazurin to provide insight into the wells of interest, which were further investigated.

The interactions between various concentrations of the antibiotics ciprofloxacin and ampicillin with nickel were assessed by the fractional inhibitory concentrations (FICs). FIC was calculated in the following manner:

$$\text{FIC of agent A} = \frac{\text{MIC of agent A in combination}}{\text{MIC of agent A}}$$

$$\text{FIC of agent B} = \frac{\text{MIC of agent B in combination}}{\text{MIC of agent B}}$$

$$\Sigma\text{FIC} = \text{FIC of agent A} + \text{FIC of agent B}$$

Interaction was defined as synergic when $\Sigma\text{FIC} < 0.5$, additive when $0.5 \leq \Sigma\text{FIC} \leq 1$, indifferent when $1 < \Sigma\text{FIC} \leq 4$ and antagonistic when $\Sigma\text{FIC} > 4$ (Tserennadmid et al. 2011).

Growth curve assay for the determination of the interaction of metals and antibiotics

After screening with the resazurin checkerboard method, new checkerboard plates were prepared as previously described and incubated in a microplate reader (Microplate Reader ELX 808, Biotek, Vermont, USA) for 24 h to obtain growth curves with measurements of optical density (OD) every hour. Measurements were performed in triplicate. Growth curves of bacteria not exposed to nickel and antibiotics were compared to those exposed to various combinations of different concentrations.

The exponential (log) phase of bacterial growth was evaluated by constructing a logarithmic line of the phase and determining the linear function $y = ax + b$, where "a" is the slope of the line, i.e., the growth rate or speed of the log phase. ANOVA and the Student–Neuman–Keuls test were performed for statistical analysis. The effect size, i.e., the magnitude of the difference between the experimental conditions and the amount of variability accounting for the experimental conditions, was assessed by r, R^2 and η^2. The following criteria were used for interpretation: $r < 0.1$ = insignificant effect size, 0.1–0.3 = small effect size, 0.3–0.5 = medium, 0.5–0.7 = large and > 0.7 very large effect size; for squared values R^2 and η^2, 0.02–0.13 = small effect size, 0.13–0.26 = medium and > 0.26 = large effect size (Rosenthal 1996). Statistical data were obtained using commercial statistical software IBM SPSS (IBM Corp, Armonk, NY, SPSS).

Results

MIC of ampicillin, ciprofloxacin and Ni

The MIC for ampicillin was 1 mg/L for *S. aureus* and *E. faecalis* and 2.5 mg/L for *E. coli*. The MICs for ciprofloxacin obtained for *E. faecalis* were 0.31 mg/L and 0.62 mg/L for *S. aureus* and 0.005 for *E. coli*. The MIC for nickel was 1000 mg/L for all bacteria.

Table 1 FIC values for ciprofloxacin and nickel

S. aureus	Concentrations (mg/L)	Ciprofloxacin	**0,62**	**0,62**	**0,62**	**0,31**	**0,31**	**0,31**	**0,16**	**0,16**	**0,16**
		Ni	**125**	**250**	500	125	250	500	125	250	500
		FIC_{CIP}	**0,5**	**0,5**	1	0,5	0,5	1	0,5	0,5	1
		FIC_{Ni}	**0,5**	**0,5**	0,5	1	1	1	1	1	1
		ΣFIC	**1**	**1**	1,5	1,5	1,5	2	1,5	1,5	2
		Interaction	**Ad**	**Ad**	Ind	Ind	Ind	Ind	Ind	Ind	Ind
E. faecalis	Concentrations (mg/L)	Ciprofloxacin	0,62	0,62	**0,62**	0,31	0,31	**0,31**	0,16	0,16	**0,16**
		Ni	125	250	**500**	125	250	**500**	125	250	**500**
		FIC_{CIP}	1	2	**4**	1	2	**4**	1	2	**4**
		FIC_{Ni}	1	1	**1**	1	1	**1**	1	1	**1**
		ΣFIC	2	3	**5**	2	3	**5**	2	3	**5**
		Interaction	Ind	Ind	**Ant**	Ind	Ind	**Ant**	Ind	Ind	**Ant**
E. coli	Concentrations (mg/L)	Ciprofloxacin	0,01	0,01	0,01	0,005	0,005	0,005	0,00025	0,00025	0,00025
		Ni	125	250	500	125	250	500	125	250	500
		FIC CIP	1	2	**4**	1	2	**4**	1	2	**4**
		FIC Ni	1	1	**1**	1	1	**1**	1	1	**1**
		ΣFIC	2	3	**5**	2	3	**5**	2	3	**5**
		Interaction	Ind	Ind	**Ant**	Ind	Ind	**Ant**	Ind	Ind	**Ant**

FIC fractional inhibitory concentration, *ΣFIC* sum of FICs for antibiotic and nickel. Interaction is defined as indifferent (Ind), additive (Ad), synergistic (Sin) or antagonistic (Ant); interactions that are not indifferent are shown in bold

ΣFIC

The ΣFIC values for all three bacteria are presented in Table 1 for ciprofloxacin and Table 2 for ampicillin.

Growth rates of *S. aureus*

The concentration of ciprofloxacin significantly decreased the growth rate of *S. aureus* with a very large effect size and a threshold concentration of 0.62 mg/L in comparison to 0.31 mg/L (0.037 ± 0.032 vs. $0.149 \pm 0{,}024$; $p < 0.001$; $\eta^2 = 0.938$). At a nickel concentrations of 500 mg/L, 1.25 mg/L ciprofloxacin blocked the growth rate in comparison to 0.62 mg/L and lower ($p < 0.001$; $\eta^2 = 0.873$), while at nickel concentrations of 125 and 250 mg/L, 0.31 mg/L ciprofloxacin decelerated the growth compared to lower concentrations starting with 0.16 mg/L (0.068 ± 0.054 vs. 0.156 ± 0.048 and 0.087 ± 0.059 vs. 0.158 ± 0.034, $p < 0.001$; $\eta^2 = 0.890$ and 0.913, respectively). There was no bacterial growth at 1000 and 2000 mg/L nickel.

Regarding ampicillin, the concentration that significantly decreased the growth rate in comparison to lower concentrations was 2 µg/mL with a very large effect size (0 ± 0 vs. 0.132 ± 0.029; $p < 0.001$; $\eta^2 = 0.898$). At a nickel concentration of 125 µg/mL, 1 µg/mL ampicillin was the threshold concentration that significantly decreased the growth rate (0.102 ± 0.088 vs. 0.200 ± 0.019, $p < 0.001$; $\eta^2 = 0.871$).

Growth rates of *E. faecalis*

The concentration of ciprofloxacin was a significant decelerator of the acceleration phase of the growth of *E. faecalis*, with a very large effect size and a threshold concentration of 0.31 mg/L (0.043 ± 0.075; $p < 0.001$; $\eta^2 = 0.729$). That concentration did not differ significantly from 0.16 and 0.08, but it did differ from bacteria without ciprofloxacin (0.177 ± 0.005). At a nickel concentration of 250 mg/L, the concentration of ciprofloxacin was a significant decelerator of growth with a large effect size ($p = 0.003$; $\eta^2 = 0.703$), with a concentration of 0.62 blocking growth. At nickel concentrations of 125 and 500 mg/L, there were no detected threshold concentrations of ciprofloxacin that significantly decelerated the growth rate in comparison to other concentrations.

The concentration of ampicillin that significantly decreased the growth rate in comparison to lower concentrations was 1 mg/L (0.052 ± 0.090 vs. 0.182 ± 0.020; $p < 0.001$; $\eta^2 = 0.886$). At a nickel concentration of 125 mg/L, the concentration of ampicillin was 0.5 mg/L (0.0 ± 0.0 vs. 0.177 ± 0.71, $p < 0.001$, $\eta^2 = 0.908$). At 250 mg/L nickel, the threshold concentration of ampicillin was 0.5 mg/L, and at 500 mg/L nickel, the threshold concentration was 0.25 mg/L ampicillin ($p < 0.001$, $\eta^2 = 0.980$).

Growth rates of *E. coli*

The threshold concentration of ciprofloxacin that significantly decreased the growth rate of *E. coli* was 0.005 mg/L

Table 2 FIC values for ampicillin and nickel

S. aureus	Concentrations (mg/L)	Ampicillin	**1**	**1**	**1**	**0,5**	**0,5**	**0,5**	**0,25**	**0,25**	**0,25**
		Ni	125	250	500	125	250	500	125	250	500
		FIC_{CIP}	1	1	0,5	1	1	0,5	1	1	0,5
		FIC_{Ni}	1	1	1	1	1	1	1	1	1
		ΣFIC	2	2	1,5	2	2	1,5	2	2	1,5
		Interaction	Ind	Ind	Ind	Ind	Ind	Ind	Ind	Ind	Ind
E. faecalis	Concentrations (mg/L)	Ampicillin	1	1	1	0,5	0,5	0,5	0,25	0,25	**0,16**
		Ni	125	250	500	125	250	500	125	250	**500**
		FIC_{CIP}	**0,25**	**0,25**	**0,1**	**0,25**	**0,25**	**0,1**	0,25	0,25	**4**
		FIC_{Ni}	**0,25**	**0,25**	**0,25**	**0,25**	**0,25**	**0,25**	1	1	**1**
		ΣFIC	**0,5**	**0,5**	**0,35**	**0,5**	**0,5**	**0,35**	1,25	1,25	**5**
		Interaction	**Ad**	**Ad**	**Sin**	**Ad**	**Ad**	**Sin**	Ind	Ind	**Ant**
E. coli	Concentrations (mg/L)	Ampicillin	2,5	2,5	2,5	1,25	1,25	1,25	0,62	0,62	0,00025
		Ni	125	250	500	125	250	500	125	250	500
		FIK AMP	1	1	**0,5**	1	1	0,5	1	1	**4**
		FIK Ni	0,5	0,5	**0,5**	1	1	1	1	1	**1**
		ΣFIK	1,5	1,5	**1**	2	2	1,5	2	2	**5**
		Interaction	Ind	Ind	**Ad**	Ind	Ind	Ind	Ind	Ind	**Ant**

FIC fractional inhibitory concentration, *ΣFIC* sum of FICs for antibiotic and nickel. Interaction is defined as indifferent (Ind), additive (Ad), synergistic (Sin) or antagonistic (Ant); interactions that are not indifferent are shown in bold

($p < 0.001$; $\eta^2 = 0.998$). When combined with nickel concentrations of 125 mg/L and 250 mg/L, the concentration of 0.005 mg/L ciprofloxacin was again the threshold. At 500 mg/L nickel, the threshold concentration that significantly decreased the growth rates in comparison to the lower concentration was 0.04 mg/L ciprofloxacin ($p < 0.001$; $\eta^2 = 0.680$).

For ampicillin, the threshold concentration was 2.5 mg/L with a large effect size (0.075 ± 0.048 vs. 0.230 ± 0.030; $p < 0.001$; $\eta^2 = 0.959$). In 125 and 250 mg/L nickel, the threshold concentration of ampicillin was the same ($p < 0.001$, $\eta^2 = 0.970$ and $\eta^2 = 0.986$).

Growth curves OD/t

The growth curves of bacteria demonstrated the synergistic effect of 2.5 mg/L ampicillin and 500 mg/L nickel on *E. coli.* Similar results were observed for *E. faecalis* at concentrations of 1 mg/L ampicillin and 500 mg/L nickel (Fig. 1). The growth curves of *S. aureus* showed no synergism or antagonism between ampicillin and nickel.

At concentrations of 0.31 mg/L ciprofloxacin and 125 mg/L nickel, a synergistic effect was observed on the growth curves of *S. aureus* (Fig. 2). Antagonistic effects were seen for ciprofloxacin and nickel on the bacterial growth of *E. faecalis* and *E. coli*. For *E. coli*, a nickel concentration of 250 mg/L acted antagonistically on 0.005 mg/L ciprofloxacin (Fig. 2). The same was observed in *E. faecalis* for 500 mg/L nickel and 0.62 mg/L ciprofloxacin (Fig. 2).

Discussion

The MIC for ciprofloxacin obtained for *S. aureus* was slightly higher than the values according to EUCAST, which were in the range of 0.125–0.5 mg/L, although a different method was used. The inhibitory concentrations for E. *faecalis* and *E. coli* were in concordance with EUCAST, where ranges of 0.25–2 mg/L and 0.004–0.016 mg/L were reported. The MICs for ampicillin against *E. faecalis* and *E. coli* were consistent with the EUCAST ranges of 0.5–2 and 2–8 mg/L, respectively (European Committee on Antimicrobial Susceptibility Testing 2019).

This study found similar inhibitory concentrations of nickel to those in previous research performed on multiple bacteria. The range of nickel minimum inhibitory concentrations was 512–1024 mg/L. The bacteria tested were enterobacteria, nonfermenting gram-negative rods and gram-positive cocci from water samples (Resende et al. 2012).

The growth rates of *S. aureus* made it evident that the concentration of ciprofloxacin decelerates the exponential phase of growth, with the concentration of 0.62 mg/L being the threshold for significant deceleration, confirming the MIC results. When 125 or 250 mg/L nickel was present, the threshold deteriorated to 0.32 mg/L ciprofloxacin. It seems that some concentrations of Ni might have a synergistic effect with ciprofloxacin on the growth rate of *S. aureus*. Nickel concentration also had a significant effect on the exponential phase of growth. Although some synergism was statistically observed, the FIC results demonstrated no

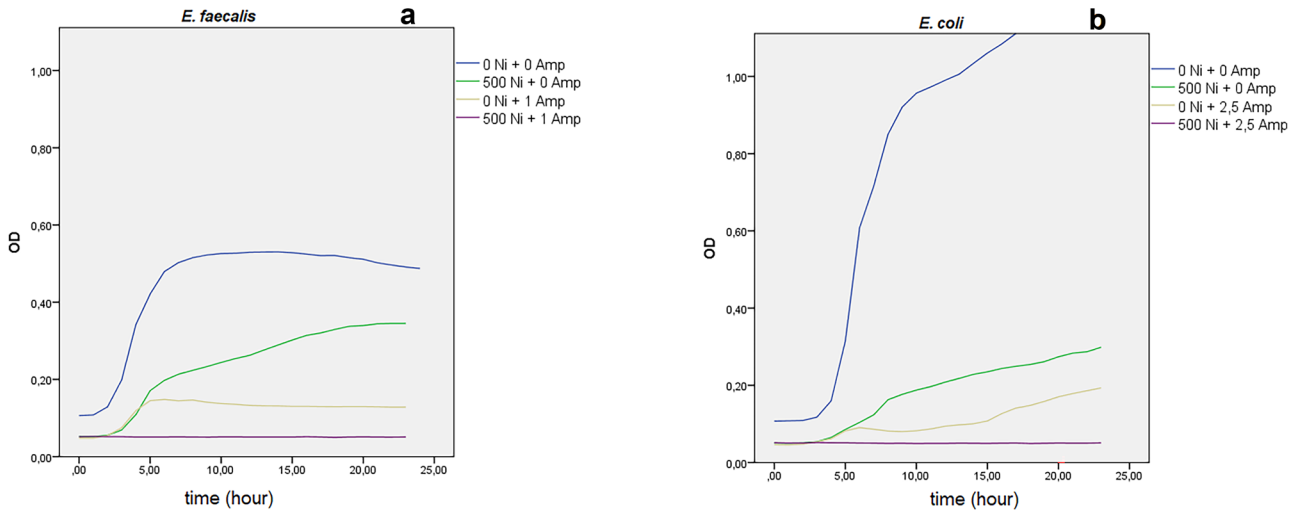

Fig. 1 Growth curves of *E. coli* and *E. faecalis* in different ampicillin and nickel concentrations (Ni, nickel - concentrations in mg/L - 0 and 500; Amp, ampicillin - concentrations in mg/L - 0, 1 and 2.5; curve displays mean values of optical density - OD at 600 nm throughout 24 h, measured in triplicate)

synergism or antagonism between ciprofloxacin and nickel on *S. aureus* growth. A similar result was found in a study that evaluated the synergistic effect of bismuth sulfide and ciprofloxacin (Ma et al. 2017).

Ampicillin and nickel had a synergistic effect on the growth of *E. faecalis* and *E. coli* with no effect seen on the growth of *S. aureus*. A study from 2016 demonstrated a synergistic effect of zinc oxide and ampicillin on an array of bacterial strains (Sharma et al. 2016). A synergistic effect of beta lactam antibiotics and ions of copper, silver and zinc was also observed (Möhler et al. 2017).

The ciprofloxacin concentration decelerated the growth of *E. faecalis*, as did the nickel concentration. A concentration of 0.31 mg/L was the cut-off that decelerated exponential growth when no nickel was added.

Interestingly, the FIC results showed an antagonistic relationship of the antibiotic and metal in *E. faecalis* and *E. coli*, which is most pronounced at a nickel concentration of 500 mg/L. Growth curves corroborate these results.

There are two main explanations for this phenomenon. One is that the chelation of metals and antibiotics invokes a decreased antimicrobial effect (Chen et al. 2015). The second is the occurrence of bacterial resistance, as it has been shown that the presence of heavy metals stimulates the expression of genes in bacteria that are responsible for various mechanisms of antimicrobial resistance. Specifically, bacteria that become resistant to nickel demonstrate the activation of efflux pumps, the elimination of nickel via vacuoles and the production of nickel sulfides to decrease the concentration of nickel in nearby surroundings (Macomber and Hausinger 2011). Efflux pumps are a significant contributor to resistance to fluoroquinolones, especially in strains demonstrating very elevated MIC values (Everett et al. 1996).

The results of this study might imply several interesting phenomena. First, nickel has an antagonistic effect on antibiotics. Further research might elucidate whether elevated nickel intake or the long-term presence of dental devices and appliances that corrode might lead to interference with antibiotic treatment. Second, the highest antagonism of metal against antibiotics was at a subinhibitory concentration of nickel, 500 mg/L. The importance of subinhibitory concentrations of heavy metals and antibiotics as a promotor of resistance development has already been emphasised in scientific evidence (Zhang et al. 2018; Bhattacharya et al. 2017).

Additionally, it seems that bacterial type plays a role in the mutual effect of nickel and ciprofloxacin, which additionally implies that the bacterial resistance mechanism could be the reason for diminished antimicrobial susceptibility. However, one can hypothesise that the 24-h incubation performed in this study might not be enough for the occurrence of this phenomenon.

In vitro studies that measured the levels of nickel in saliva reported a concentration of 0.414 mg/L released from Ni–Cr dental alloys (Reclaru et al. 2012), 0.0269 ppm/day from stainless steel dental crowns (Ramazani et al. 2014) and 27.04 µg/day from orthodontic wires (Katic et al. 2017). In vivo studies show salivary levels of nickel in orthodontic patients to be 0.0853 mg/L (Gölz et al. 2016), and nickel found in the oral mucosa cells of orthodontic patients ranged up to 0.0217 mg/L (Downarowicz and Mikulewicz 2017). Although these concentrations are significantly lower than those causing interactions with ciprofloxacin or bacteria, it is difficult to assess the cumulative concentration to which bacteria could be exposed. Additionally,

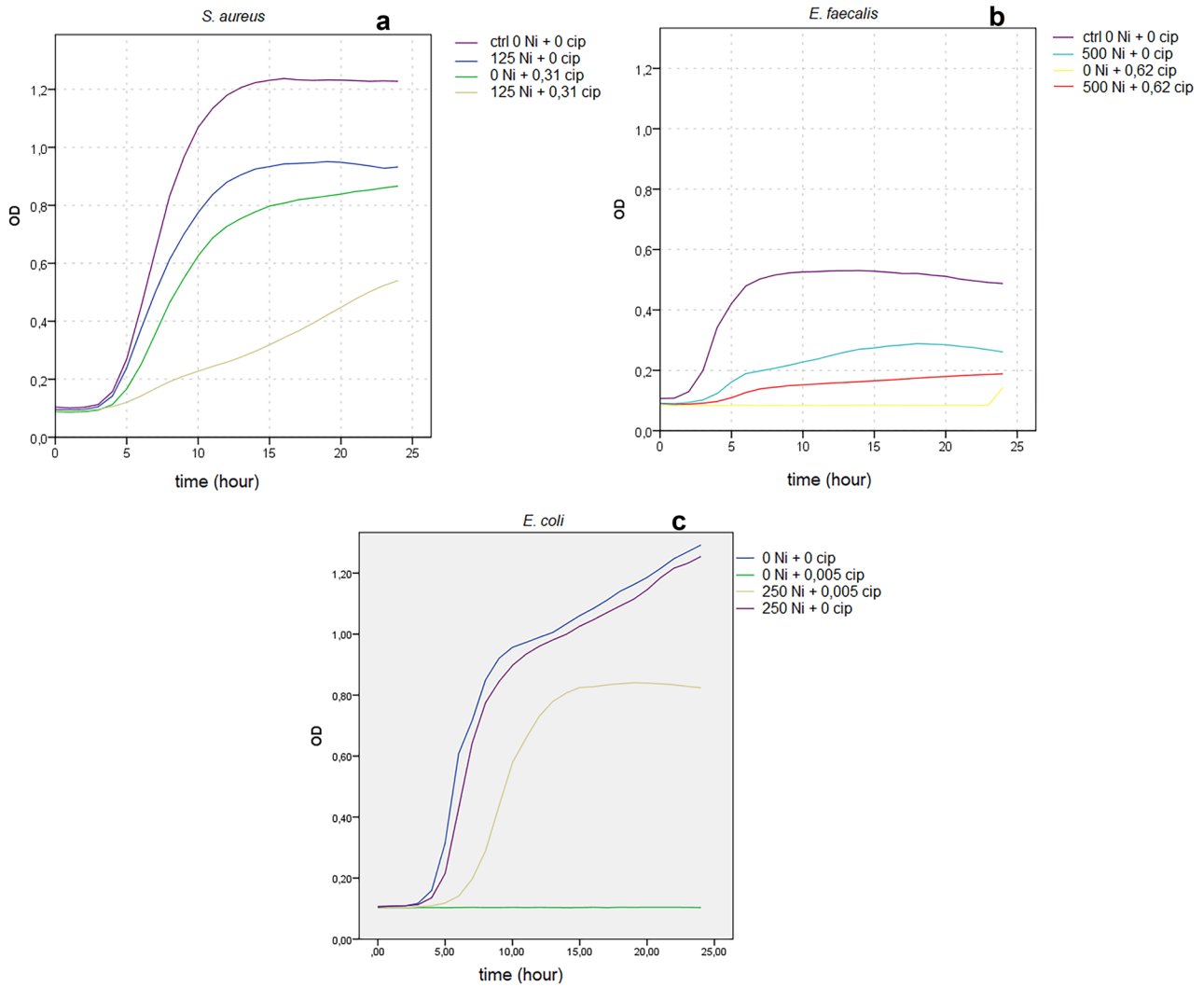

Fig. 2 Growth curves of *S. aureus*, *E. faecalis* and *E. coli* and in different ciprofloxacin and nickel concentrations (Ni, nickel - concentrations in 0, 125 and 500; cip, ciprofloxacin - concentrations in 0, 0.31 and 0.62; curve displays mean values of optical density - OD at 600 nm throughout 24 h, measured in triplicate)

it should be emphasised that orthodontic treatment lasts approximately 2.5 years, during which time appliances remain in the oral cavity and release nickel ions by corrosion (Wang et al. 2018). Perhaps a collection of commensal bacteria in patients with dental devices and orthodontic appliances could provide an answer.

Overall, it seems that nickel could have repercussions on the human body more important than the direct effect on human cells, namely, its influence on the human microbiota and interference with antibiotics. Additionally, nickel in the body could interfere with the uptake of antibiotics, although further research is necessary to elucidate the causal inference.

A study with longer exposure of bacteria is definitely needed to confirm the potential adaptation to nickel and the development of resistance mechanisms.

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Availability of data and materials Data are available.

Declarations

Conflict of interest The authors declare no competing interests.

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