










Tolerance to disinfectants (chlorhexidine and isopropanol) and its association with antibiotic resistance in clinically-related *Klebsiella pneumoniae* isolates

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ABSTRACT

Disinfectants play an essential role in controlling the dissemination of bacteria in health care settings, but it may also contribute to the selection of antibiotic resistance bacteria. This study looked at *Klebsiella pneumoniae* isolates collected from three hospitals in Lima, Peru, in order to evaluate: their susceptibility to chlorhexidine [CHG] and isopropanol [ISP]), and their association with antimicrobial susceptibility. We analyzed 59 *K. pneumoniae* isolates and assessed their CHG and ISP susceptibility by minimum inhibitory concentrations (MICs). Additionally, we performed a regression analysis to assess the association between disinfectant tolerance and antibiotic resistance (measured by the disc diffusion method), colistin resistance (by microdilution), carbapenemases presence (by polymerase chain reaction [PCR]), and clonal relationships (by pulsed-field gel electrophoresis [PFGE]). Eleven *K. pneumoniae* strains were isolated from fomites, and 48 strains from clinical samples. The MIC range of these isolates was 8–128 µg/ml for CHG and 16–256 mg/ml for ISP. We found that resistance to trimethoprim/sulfamethoxazole (TMP/SMX) was the main factor associated with CHG log₂ MIC ($\beta = 0.65$; 95%CI: 0.03, 1.27; $R^2 = 0.07$). In the case of ISP, the log₂(MIC) was associated with the institution of origin, showing lower ISP log₂(MIC) in fomites compared to clinical samples ($\beta = -0.77$; 95%CI: -1.54, -0.01; $R^2 = 0.08$). Resistance to CHG and ISP among *K. pneumoniae* isolates found in Peruvian hospitals seems to be elevated and highly variable. Further studies are needed to confirm our results and implement actionable interventions if necessary.

KEYWORDS

Disinfectants; chlorhexidine; alcohol-based disinfectants; *Klebsiella pneumoniae*; cross-resistance; antibiotic resistance

Introduction

Biocides are routinely used in healthcare settings and play an essential role in infection control [1], one that has been highlighted during the fight against SARS-CoV-2. Two of the most frequently used types of biocides in hospitals are chlorhexidine digluconate (CHG) and isopropyl alcohol (ISP). Chlorhexidine is a bisbiguanide antiseptic used against a wide range of microorganisms. It functions by forming a bridge between phospholipids with subsequent displacement of cations (Mg²⁺ and Ca²⁺) [2], resulting in membrane disruption via a reduction in the capacity of the bacterial membrane to osmoregulate and changes in enzymes associated with metabolic membrane capability [2]. Chlorhexidine is often used for procedures such as handwashing, preoperative preparation, and surface disinfection because of its bacteriostatic and bactericidal properties [3,4], as well as its ability to rapidly denature the proteins of microorganisms [5].

In recent decades, infections associated with multi-drug-resistant (MDR) pathogens have increased, being

Klebsiella pneumoniae one of the most resistant species. This is of concern because *K. pneumoniae* has also been associated with high mortality rates and an increased use of last-resort antibiotics, such as carbapenems, colistin, and their combinations [6]. These MDR pathogens are responsible for increasing mortality rates secondary to hospital-acquired infections, readmissions, and emergency department visits after discharge [7].

There is an ongoing debate about whether there is an association between an increased tolerance of this bacteria to disinfectants and its resistance to antibiotics. There is evidence suggesting that hygiene promotion, which includes biocides use, helps prevent bacteria transmission across health care settings, although further research is needed to determine the consequence of biocides uses on the appearance or selection of antibiotic resistance bacteria [8,9]. This study aimed to test the levels of tolerance of *K. pneumoniae* strains to CHG and ISP and determine their association with antimicrobial resistance in Peruvian health care settings before COVID-19.

Materials and methods

Bacterial strains

The *Klebsiella* spp. strains evaluated were obtained from bacterial collections at the Molecular Microbiology Laboratory of the *Universidad Científica del Sur* in Lima, Peru. The *K. pneumoniae* isolates were from 3 different Institutions in Lima: the *Hospital Nacional 2 de Mayo* (HN2M) (fomites isolates collected from clinical mobile phones and uniforms), as well as *Instituto Nacional Materno Perinatal (INMP)* and *Hospital Nacional Guillermo Almenara Irigoyen (HNGAI)*, both of which provided clinical isolates (bacteremia, secretions). The strains were collected in 2018, and the identification was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS).

This study was approved by the Ethical Committee of the *Universidad Científica del Sur* in Lima, Peru.

Preparation of disinfectant (chlorhexidine and isopropanol) solutions

Stock solutions of CHG (CHG; 40 mg/mL) were prepared in sterile distilled water at a concentration of 768 µg/ml prior to further dilution (256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 µg/ml) in Mueller Hinton broth and distributed into 96-well plates. Stock solutions of ISP (ISO; EMSURE®) were prepared at 768 mg/L in sterile distilled water and were also diluted (256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 mg/ml) in Mueller Hinton broth and placed in 96-well plates. We used the strains ATCC 13883 *Klebsiella pneumoniae* as a control. The disinfectants were added separately to these strains and used as a positive control. For negative control, only the culture media was used to monitor the sterility.

Disinfectant susceptibility testing

Minimum inhibitory concentrations (MICs) were determined using the Mueller Hinton broth microdilution method established by the Clinical Laboratory Standards Institute (CLSI) [10]. Briefly, bacterial strains were resuspended in phosphate-buffered saline (PBS) 0.5x to a 0.5 McFarland concentration. Lecture was done after 18–20 hours of incubation at 37°C. The MIC was defined as the lowest concentration of the disinfectant agents that inhibits the visible growth of the isolate tested observed with the unaided eye. In the absence of an established cutoff, we defined MIC₅₀ as the MIC necessary to inhibit the growth of 50% of the study isolates, while MIC₉₀ was considered the MIC necessary to inhibit the growth of 90% of the study isolates.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by a conventional disc diffusion method. The

antimicrobials included were meropenem, imipenem, piperacillin/tazobactam, chloramphenicol, trimethoprim/sulfamethoxazole, nitrofurantoin, levofloxacin, ciprofloxacin, aztreonam, cefotaxime, amoxicillin plus clavulanic acid, gentamicin, and amikacin. The category of resistance was interpreted according to CLSI guidelines [10]. The *Escherichia coli* strain ATCC 25922 was used as a control. Screening for colistin was evaluated by microdilution as recommended by the CLSI-EUCAST Polymyxin Breakpoints Working Group in order to describe MICs [11].

Presence of carbapenemases

The presence of carbapenemases was evaluated in isolates with diminished carbapenem susceptibility. Thus, these strains were screened by a multiplex polymerase chain reaction (PCR) test for the *bla*_{KPC}, *bla*_{OXA-48}, *bla*_{VIM}, *bla*_{IMP}, and *bla*_{NDM} genes [12].

Clonality studies

The isolates' clonal relatedness was determined by pulsed-field gel electrophoresis (PFGE), as described previously [13]. Band patterns were analyzed using InfoQuest™ FP v.5.4 software. Bacteria with a similarity > 80% were considered to be clonally related.

Statistical analysis

Descriptive analysis of disinfectant susceptibility was performed using the MICs 50, MIC 90, and CHG and ISP range separately. We conducted a bivariate analysis using the Kruskal Wallis test for means log₂(MIC) comparisons. We estimated the magnitude of association modeling the log₂(MIC) using generalized linear models, a Gaussian family, link identity, and robust variances. During this process, we included the following variables as factors potentially associated with CHG and ISP log₂(MIC), separately: institution and source of isolate origin, carbapenemases positivity, clonality, and resistance to common and last-resort antibiotics. In all cases, we estimated 95% confidence intervals (95% CI), and the STATA MP v14.0 statistical package (Stata Corp LP, College Station, Texas) was used for the statistical analyses. We used a p-value of 0.05 to measure the statistical significance and a p-value of 0,10 for exploratory analysis.

Results

Bacterial strains

A total of 59 *K. pneumoniae* isolates were included in the study. Among these, 11 (18.6%) were isolated from HN2M, 7 (64%) were isolated from mobile phones, and 4 (36%) were isolated from the

clothing of health care workers. 23 (38.9%) were clinical isolates from blood samples of bacteremia from the HNGAI, and 25 (42.3%) were from blood and secretions of patients from the INMP. All the isolates belonged to a collection obtained in 2018 and stored at -80°C in the *Universidad Científica del Sur* Microbiology Laboratory.

Antibiotic susceptibility

The *K. pneumoniae* collection that was studied presented a high rate of resistance to ciprofloxacin, amoxicillin plus clavulanic acid, ceftriaxone, and ceftazidime (86.4, 72.4, 74.6, and 75.8% respectively). Resistance rates of nearly 60% were found to aztreonam, trimethoprim/sulfamethoxazole, and nitrofurantoin (60.7%, 60.3%, and 66.1%, respectively), 51.9% to piperacillin/tazobactam, 51.7% to both imipenem and chloramphenicol, 42.1% to levofloxacin, 48.2% to meropenem, and 45.6% to gentamicin. Finally, low resistance levels to amikacin (36.2%) and colistin (8.5%) were reported.

Disinfectant susceptibility

When analyzing CHG resistance, we observed high variability of MICs (coefficient of variation [CV] = 0.81), ranging from 8 to 128 $\mu\text{g/ml}$ (Figure 1). The MIC₅₀ of all the isolates included in the study was 32 $\mu\text{g/ml}$. In the bivariate analysis, we found marginal MIC differences between CHG isolates testing positive versus negative to carbapenemases and resistant to levofloxacin. In contrast, statistically significant differences were found between those testing positive versus negative to resistance to TMP/SMX (Tables 1 and 2). On the other hand, when analyzing ISP resistance, we also observed a wide range of MICs against ISP (CV = 0.80), 16–256 mg/ml (Figure 1). The ISP MIC₅₀ of all the isolates was 64 mg/ml

ml. In the bivariate analysis, we found significant differences among sample sources ($p = 0.04$), and marginal differences among institutions ($p = 0.08$). Specifically, we observed a lower MIC₅₀ and MIC₉₀ among HN2M isolates (32 and 64 mg/ml, respectively) compared to those obtained from HNGAI (64 and 128 mg/ml, respectively) and INMP (64 and 128 mg/ml, respectively). Likewise, we observed a lower MIC₅₀ and MIC₉₀ among isolates from fomites from health workers' mobile phones/clothing (32 and 64 mg/ml, respectively) compared to those from blood and secretions (64 and 128 mg/ml, respectively) (Table 1).

Presence of carbapenemases

The isolates that presented a resistance phenotype to carbapenems (either imipenem or meropenem) were tested by PCR, detecting the *bla*_{NDM} gene in 23 of them (38.9%). We did not find other carbapenemases genes, such as *bla*_{KPC}, *bla*_{Oxa-48}, *bla*_{IMP}, or *bla*_{VIM}.

Clonality analysis

When testing PFGE patterns, we observed that all the isolates belonged to different PFGE types, except for 20 strains classified in the same group. Despite these results, we did not observe significant or marginal differences in the MICs of CHG and ISP compared with the rest of the isolates.

Association of antibiotic resistance with disinfectant susceptibility

In the bivariate regression analysis, a significant direct association was found between the CHGlog₂(MIC) and resistance to TMP/SMX ($p = 0.041$). Also, marginal direct associations were observed with positivity to carbapenemases ($p = 0.072$) and resistance to

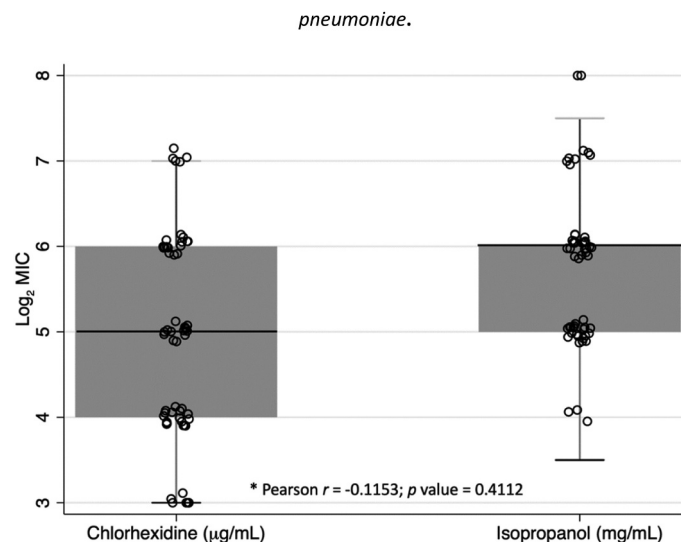


Figure 1. Log₂(MIC) of chlorhexidine and isopropanol in strains of *Klebsiella pneumoniae*.

Table 1. MICs of chlorhexidine and isopropanol by the study sample's characteristics.

Characteristic	Chlorhexidine MIC ($\mu\text{g/mL}$)			<i>p</i> -value	Isopropanol MIC (mg/mL)			<i>p</i> -value
	MIC ₅₀	MIC ₉₀	Range		MIC ₅₀	MIC ₉₀	Range	
All	32	64	8–128		64	128	16–256	
Institution								
INMP	16	64	8–128	0.123	64	128	32–256	0.084†
HNGAI	64	128	8–128		64	128	16–256	
HN2M	32	64	16–64		32	64	32–64	
Sample source								
Fomite	32	64	16–64	0.541	32	64	32–64	0.038‡
Blood/Secretions	32	128	8–128		64	128	16–256	
Clonality								
Negative	16	64	8–128	0.192	64	128	16–64	0.239
Positive	64		16–256		32		32–64	
Carbapenemases								
Absence	16	64	8–128	0.071†	64	128	32–256	0.455
Presence	64	128	8–128		64	128	16–256	

MIC, Minimal inhibitory concentration; INMP, Instituto Nacional Materno Perinatal; HNGAI, Hospital Nacional Guillermo Almenara Irigoyen; HN2M, Hospital Nacional Dos de Mayo; †, *p* value <0.10; ‡, *p* value <0.05

Table 2. MICs of chlorhexidine and isopropanol by antibiotics.

Antimicrobial agents	Chlorhexidine MIC ($\mu\text{g/mL}$)			<i>p</i> -value	Isopropanol MIC (mg/mL)			<i>p</i> -value
	MIC ₅₀	MIC ₉₀	Range		MIC ₅₀	MIC ₉₀	Range	
Colistin								
Sensitive	32	64	8–128	0.329	64	128	16–256	0.609
Resistant	32	32	8–32		80	128	32–128	
Imipenem								
Sensitive	32	64	8–128	0.105	64	128	32–128	0.675
Resistant	32	128	8–128		64	128	16–256	
Meropenem								
Sensitive	32	64	8–128	0.158	64	128	32–256	0.647
Resistant	32	128	8–128		64	128	16–256	
PIP/TZB								
Sensitive	32	64	8–128	0.165	64	128	32–128	0.815
Resistant	32	128	8–128		64	128	16–128	
Chloramphenicol								
Sensitive	32	128	8–128	0.183	32	128	16–256	0.101
Resistant	16	64	32–256		64	128	32–256	
TMP/SMX								
Sensitive	16	64	8–128	0.039‡	64	128	32–128	0.708
Resistant	32	128	8–128		64	128	16–256	
Nitrofurantoin								
Sensitive	32	64	8–64	0.875	48	128	16–128	0.610
Resistant	32	128	8–128		64	128	16–256	
Aztreonam								
Sensitive	32	64	8–128	0.598	32	128	32–256	0.510
Resistant	32	64	8–128		64	128	16–256	
Ciprofloxacin								
Sensitive	64	128	8–128	0.138	48	256	32–256	0.697
Resistant	32	64	8–128		64	128	16–256	
Levofloxacin								
Sensitive	32	128	8–128	0.059†	64	128	16–256	0.596
Resistant	16	64	8–128		64	128	32–128	
Cefotaxime								
Sensitive	32	128	8–128	0.979	64	128	32–256	0.991
Resistant	32	64	8–128		64	128	16–256	
Ceftazidime								
Sensitive	32	64	8–64	0.881	64	64	32–128	0.781
Resistant	32	128	8–128		64	128	16–256	
AMC								
Sensitive	16	64	8–128	0.266	64	256	32–256	0.199
Resistant	32	64	8–128		64	128	16–128	
Gentamicin								
Sensitive	32	64	8–128	0.115	64	128	32–256	0.586
Resistant	64	128	8–128		64	128	16–128	
Amikacin								
Sensitive	-			NA	-			NA
Resistant	32	64	8–128		64	128	16–256	

MIC, Minimal Inhibitory Concentration; Me, Median; IQR, Interquartile range; Pip/tzb, piperacillin/tazobactam; TMP/SMX, trimethoprim/sulfamethoxazole; AMC, amoxicillin/clavulanate; †, *p* value <0.10; ‡, *p* value <0.05

imipenem ($p = 0.094$) and levofloxacin ($p = 0.061$) (Table 3). However, in the multivariate regression analysis, the only resistance to TMP/SMX was observed as the main predictor of the CHG $\log_2(\text{MIC})$ ($\beta = 0.65$; 95%

CI: 0.03, 1.27; $R^2 = 0.1127$). On the other hand, when modeling the ISP $\log_2(\text{MIC})$, we observed a significant association with the institution of origin of the sample, with HN2M samples showing a lower ISP $\log_2(\text{MIC})$

Table 3. Regression analysis for log₂(MIC) of chlorhexidine and isopropanol.

Associated Factor	Chlorhexidine MIC		Isopropanol MIC	
	β (95% CI)	p-value	β (95% CI)	p-value
Institution				
INMP	Ref.		Ref.	
HNGAI	0.66 (−0.01, 1.32)	0.054†	−0.23 (−0.76, 0.29)	0.375
HN2M	0.53 (−0.31, 1.37)	0.210	−0.77 (−1.54, −0.01)	0.048‡
Fomites source	−0.22 (−1.01, 0.58)	0.588	−0.22 (−1.01, 0.58)	0.588
PFGE Type 4	0.51 (−0.26, 1.28)	0.191	−0.11 (−0.63, 0.41)	0.680
Carbapenemases	0.66 (−0.06, 1.38)	0.072†	−0.23 (−0.79, 0.32)	0.400
Colistin	−0.56 (−1.68, 0.56)	0.321	0.29 (−0.67, 1.25)	0.543
Imipenem	0.52 (−0.09, 1.13)	0.094†	−0.10 (−0.41, 0.61)	0.703
Meropenem	0.44 (−0.17, 1.06)	0.158	−0.15 (−0.66, 0.36)	0.547
PIP/TZB	0.45 (−0.16, 1.07)	0.147	−0.04 (−0.55, 0.48)	0.889
Chloramphenicol	−0.46 (−1.14, 0.23)	0.186	0.43 (−0.12, 0.98)	0.122
TMP/SMX	0.65 (0.03, 1.27)	0.041‡	−0.07 (−0.59, 0.44)	0.779
Nitrofurantoin	0.09 (−0.55, 0.75)	0.764	0.16 (−0.37, 0.69)	0.547
Aztreonam	−0.51 (−0.51, 0.81)	0.645	0.09 (−0.46, 0.63)	0.741
Ciprofloxacin	−0.68 (−1.56, 0.21)	0.132	−0.32 (−1.11, 0.47)	0.422
Levofloxacin	−0.60 (−1.23, 0.03)	0.061†	0.11 (−0.42, 0.64)	0.678
Cefotaxime	−0.02 (−0.74, 0.69)	0.946	−0.07 (−0.66, 0.52)	0.814
Ceftazidime	0.07 (−0.66, 0.81)	0.840	−0.03 (−0.53, 0.58)	0.934
AMC	0.38 (−0.30, 1.05)	0.270	−0.49 (−1.03, 0.06)	0.078
Gentamicin	0.48 (−0.15, 1.11)	0.129	−0.25 (−0.77, 0.27)	0.339

MIC, Minimal Inhibitory Concentration; INMP, Instituto Nacional Materno Perinatal; HNGAI, Hospital Nacional Guillermo Almenara Irigoyen; HN2M, Hospital Nacional Dos de Mayo; PFGE, pulsed field gel electrophoresis; PIP/TZB, piperacillin/tazobactam; TMP/SMX, trimethoprim/sulfamethoxazole; AMC, amoxicillin/clavulanate; β, linear regression coefficient; 95% CI, 95% confidence interval; †, p value <0.10; ‡, p value <0.05

compared to isolates from the INMP ($p = 0.048$). No association was observed between the ISP log₂(MIC) and any antibiotic resistance tested in the study.

Discussion

The main findings of our study include elevated and highly variable levels of resistance to CHG and ISP among clinically-related *K. pneumoniae* isolates from Peru. Additionally, we observed an association between CHG resistance and TMP/SMX resistance plus a significant variability of ISP resistance according to institution source. These results are important because both CHG and ISP are two of the main disinfectants used in health care facilities and because their use has increased substantially during COVID-19 pandemic.

Our study findings coincide with previous studies that reported a lower susceptibility of bacteria to CHG and ISP [14], which are commonly used to control the spread of *K. pneumoniae* and other bacteria and viruses in most health care settings around the world. Disinfectants are crucial to correct infection control and are extensively used in hospitals, veterinary medicine, the food industry, farms, and homes [14], but their use could associate antimicrobial resistance increase [8]. Surveillance studies are needed to evaluate the microorganisms' tolerance to disinfectants at an institutional level to establish adequate disinfection programs.

Although there are no cutoffs by international organizations such as CLSI or EUCAST to guide the interpretation of susceptibility to these substances, epidemiological cutoff values have been proposed to indicate CHG resistance in *Klebsiella* (64 µg/ml) [15]. In our study, we found a wide range of MICs of CHG in

Klebsiella isolates, with MIC₅₀ of the HNGAI samples being the highest (64 µg/ml), and according to the cutoff, they were resistant to this disinfectant. In short, our results showed 21 (35,6%) samples with MIC_{≥64} µg/ml, which, according to the epidemiological cutoff, is considered resistant. Although not statistically significant, we observed higher CHG resistance levels in the HNGAI sample compared to those from INMP, as well as among fomites (HN2M). This finding could be related to greater use of CHG in intensive care units in which patients receive daily CHG bathing, exerting selection pressure on microorganisms previously related to bacteria more tolerant to CHG [16].

We found a significant association between TMP/SMX resistance and higher levels of CHG MIC values. This association has been described in bacterial species such as *Pantoea* spp. and *Salmonella* spp., but to our knowledge, not in *K. pneumoniae* [17]. In addition, the present data demonstrate that other antibiotics have shown this association. Thus, the presence of carbapenemases is directly associated with higher CHG MIC values, and the resistance to levofloxacin has a negative association since higher CHG MIC values are related to the presence of strains susceptible to levofloxacin. Our results correlate with a previous study performed in China that suggested decreased sensitivity against commonly used disinfectants, including CHG, in carbapenem-resistant strains. Furthermore, Chen *et al.* [18] reported the presence of carbapenem-resistant *K. pneumoniae* with decreased susceptibility to CHG (MIC 32 µg/ml, which is the limit of the ecological cutoff of 64 µg/ml). It is important to mention that only six phylogenetically related strains were included in the study and all carbapenemases that were found were

*bla*_{NDM} [19]. Our data showed a negative association between fluoroquinolones and CHG resistance, while a positive association has been previously described in *Pseudomonas aeruginosa* [19]. Thus, *in vitro* exposure to CHG has been related to the selection of quinolone-resistant *P. aeruginosa* due to over-expression of the efflux pumps MexAB-OprM, MexCD-OprJ, and MexXY-OprM [20], none of which are related to *K. pneumoniae*.

We found no relationship between colistin resistance and increased tolerance to CHG compared to the study by Wand *et al.* (2017), which found that the MICs of CHG were the same in both colistin-susceptible and colistin-resistant *K. pneumoniae* strains [6]. Likewise, it has been described that the adaptation to CHG led to resistance against colistin in *K. pneumoniae* and, instead, the adaptation to colistin does not lead to resistance against CHG [6]. In *K. pneumoniae*, *in vitro* exposure to CHG increased the colistin resistance likely by induction of point mutations in *pmrBA* and *phoPQ* related to colistin resistance and/or *smvR* [21]. *SmvA* is an efflux pump belonging to the major facilitator superfamily (MFS), which has been suggested to play a major role in CHG resistance in *K. pneumoniae* strains [6].

Increasing CHG resistance has been related to the over-expression of efflux pumps involved eliminating CHG [22]. Notably, different MDR efflux systems can export both antibiotics and biocides, contributing to the co-selection of resistance to different agents. There is a gene family related to diminished CHG susceptibility called *qac* (quaternary ammonium compound) (e.g., *qacA/B* and *qacC*, among others) [23]. Of note, some *qac* genes have been shown to contribute to the increasing resistance to specific antibiotics; for instance, *QacA* and *QacBIII* contribute to the final norfloxacin and ciprofloxacin MIC levels [23]. These *qac* genes encode for Qac efflux proteins belonging to the MFS and SMR efflux pump families. While the role of these proteins is well known in Gram-positive strains, previous findings have led to controversy about the role and frequency of the *cepA*, *qacA*, and *qacE* genes in Gram-negative strains, with more or less importance being given depending on the study [18, 21, 24, 25].

Moreover, it has been described that the *qacED1* gene is located in an integron class 1 structure upstream of the *sul1* gene that confers sulfonamide resistance and is often flanked by the dihydrofolate reductase gene *dhfrA1* related to trimethoprim resistance [18]. These gene cassette structures are related to the CHG tolerance, and TMP/SMX resistance found in our study ($p < 0.05$), with both genes likely being disseminated together in the same mobile structure within the same plasmid. This relationship could be associated with the described propensity of CHG that could stimulate the horizontal transfer of antibiotic resistance [26], leading to the potential to co- and cross-select antibiotic resistance mechanisms [27].

Future studies are needed to determine the gene content in integrons inside plasmids and relate their frequency with the MIC of the biocides and diminished antimicrobial susceptibility strains.

Regarding ISP-based disinfectants, the present study found lower resistance in isolates from fomites compared to clinical isolates. This may be because strains found in potential fomites (mobile devices, health workers' clothing, etc.) are less exposed to these disinfectants than hospital surfaces.

It is worth noting that there are studies that reported cases of disinfectant-induced antibiotic resistance in bacterial strains [8,17]. This may have contributed to the antibiotic resistance in our strains if previously exposed to disinfectants in hospital settings.

No relation was found between antibiotic resistance and alcohol tolerance, which supports a previous report evidencing that no cross-resistance to antibiotics after low-level exposure to alcohol-based disinfectants has so far been described [17]. This might occur because the mechanisms of action of alcohol-based disinfectants are not related to mechanisms causing antibiotic resistance, such as inhibition and uncoupling of messenger ribonucleic acid (RNA) and protein synthesis through specific effects on RNA polymerase and ribosomes [28], or interference with cellular metabolism, disruptions of cytoplasmic integrity and cell lysis [29].

Our results showed a high proportion of CHG and ISP resistant strains. However, the clinical importance of these findings is not yet evident, since the concentrations of disinfectants used in clinical settings are, at present, greater than the highest MICs found in the study. Globally, very few studies have discussed and analyzed MIC rates of biocides together with antibiotics and their molecular mechanisms [31–33]. Antimicrobial resistance genotypes vary among different countries, regions, and hospital settings, and the same could occur with disinfectant-tolerant bacteria and their mechanisms [16]. Therefore, it is important to highlight that these results provide a baseline for measuring disinfectant susceptibility to assist in future surveillance studies in Peru.

This study evaluated pre-pandemic levels of CHG and ISP resistance. Considering the wide-scale use of biocides for personal and environmental disinfection during the COVID-19 pandemic, the current levels of resistance may be a global public health threat. Future surveillance studies are essential to understand the effect of disinfectant use and observe the possible emergence of microorganisms that can survive exposure to disinfectants.

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Disclosure statement

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References

- [1] Rutala WA. APIC guideline for selection and use of disinfectants. *Am J Infect Control*. 1996 Aug;24(4):313–342.
- [2] Hugo WB, Longworth AR. The effect of chlorhexidine on the electrophoretic mobility, cytoplasmic constituents, dehydrogenase activity and cell walls of *Escherichia coli* and *Staphylococcus aureus*. *J Pharm Pharmacol*. 1966 Sep;18(9):569–578.
- [3] Denny J, Munro CL. Chlorhexidine bathing effects on health-care-associated infections. *Biol Res Nurs*. 2017 Mar;19(2):123–136.
- [4] Lakhani N, Vandana KL. Department of periodontics, college of dental sciences, Davangere, Karnataka, India. Chlorhexidine – An insight. *Int J Adv Res*. 2016 Jul 31;4(7):1321–1328.
- [5] Boyce JM. Alcohols as surface disinfectants in health-care settings. *Infect Control Hosp Epidemiol*. 2018 Mar;39(3):323–328.
- [6] Wand ME, Bock LJ, Bonney LC, et al. Mechanisms of increased resistance to chlorhexidine and cross-resistance to colistin following exposure of *Klebsiella pneumoniae* clinical isolates to chlorhexidine. *Antimicrob Agents Chemother*. 2017 Jan;61(1):e01162–16.
- [7] Barrasa-Villar JI, Aibar-Remón C, Prieto-Andrés P, et al. Impact on morbidity, mortality, and length of stay of hospital-acquired infections by resistant microorganisms. *Clin Infect Dis*. 2017 Aug 15;65(4):644–652.
- [8] Caselli E, Brusaferrero S, Coccagna M, et al. Reducing healthcare-associated infections incidence by a probiotic-based sanitation system: A multicentre, prospective, intervention study. *PLoS One*. 2018;13(7):e0199616.
- [9] Sanchez P, Moreno E, Martinez JL. The biocide triclosan selects *Stenotrophomonas maltophilia* mutants that overproduce the SmeDEF multidrug efflux pump. *Antimicrob Agents Chemother*. 2005;49(2):781–782.
- [10] M100-S11. Performance standards for antimicrobial susceptibility testing. *Clin Microbiol Newsl*. 2001;23(6):49.
- [11] Turlej-Rogacka A, Xavier BB, Janssens L, et al. Evaluation of colistin stability in agar and comparison of four methods for MIC testing of colistin. *Eur J Clin Microbiol Infect Dis*. 2018;37(2):345–353.
- [12] Bogaerts P, Rezende de Castro R, de Mendonca R, et al. Validation of carbapenemase and extended-spectrum b-lactamase multiplex endpoint PCR assays according to ISO 15189. *J Antimicrob Chemother*. 2013;68:1576–1582.
- [13] Durmaz R, Otlu B, Koksall F, et al. The optimization of a rapid pulsed-field gel electrophoresis protocol for the typing of *Acinetobacter baumannii*, *Escherichia coli* and *Klebsiella* spp. *Jpn J Infect Dis*. 2009;62(5):372–377.
- [14] Fraiese AP. Choosing disinfectants. *J Hosp Infect*. 1999;43(4):255–264.
- [15] Morrissey I, Oggioni MR, Knight D, et al. Evaluation of epidemiological cut-off values indicates that biocide resistant subpopulations are uncommon in natural isolates of clinically-relevant microorganisms. Manganelli R, editor. *PLoS ONE*. 2014 23;9(1):e86669.
- [16] Suwantarat N, Carroll KC, Tekle T, et al. High prevalence of reduced chlorhexidine susceptibility in organisms causing central line-associated bloodstream infections. *Infect Control Hosp Epidemiol*. 2014;35(9):1183–1186.
- [17] Kampf G. Biocidal agents used for disinfection can enhance antibiotic resistance in gram-negative species. *Antibiotics (Basel)*. 2018;7(4):110.
- [18] Chen Y, Liao K, Huang Y, et al. Determining the susceptibility of carbapenem resistant *Klebsiella pneumoniae* and *Escherichia coli* strains against common disinfectants at a tertiary hospital in China. *BMC Infect Dis*. 2020;20(1):88.
- [19] Stein C, Vincze S, Kipp F, et al. Carbapenem-resistant *Klebsiella pneumoniae* with low chlorhexidine susceptibility. *Lancet Infect Dis*. 2019;19(9):932–933.
- [20] Kawamura M, Sato T, Fujimura S. In vitro exposure of chlorhexidine induce quinolone resistance in *Pseudomonas aeruginosa*. *J Infect Public Health*. 2020;13(2):317–318.
- [21] Zhang Y, Zhao Y, Xu C, et al. Chlorhexidine exposure of clinical *Klebsiella pneumoniae* strains leads to acquired resistance to this disinfectant and to colistin. *Int J Antimicrob Agents*. 2019;53(6):864–867.
- [22] Rossi L, Bertonecchi A, Tomi ED, et al. Susceptibility to chlorhexidine of *Klebsiella pneumoniae* strains: KPC producers and potential role of efflux pumps. 29th ECCMID (P2631) 2019.
- [23] Nakaminami H, Noguchi N, Sasatsu M. Fluoroquinolone efflux by the plasmid-mediated multidrug efflux pump QacB variant QacBIII in *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2010;54(10):4107–4111.
- [24] Guo W, Shan K, Xu B, et al. Determining the resistance of carbapenem-resistant *Klebsiella pneumoniae* to common disinfectants and elucidating the underlying resistance mechanisms. *Pathog Glob Health*. 2015;109(4):184–192.
- [25] Vijayakumar R, Sandle T, Al-Aboody MS, et al. Distribution of biocide resistant genes and biocides susceptibility in multidrug-resistant *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* — A first report from the Kingdom of Saudi Arabia. *J Infect Public Health*. 2018;11(6):812–816.
- [26] Jutkina J, Marathe NP, Flach C-F, et al. Antibiotics and common antibacterial biocides stimulate horizontal transfer of resistance at low concentrations. *Sci Total Environ*. 2018;616–617:172–178.
- [27] Webber MA, Whitehead RN, Mount M, et al. Parallel evolutionary pathways to antibiotic resistance

- selected by biocide exposure. *J Antimicrob Chemother.* 2015;70(8):2241–2248.
- [28] Haft RJF, Keating DH, Schwaegler T, et al. Correcting direct effects of ethanol on translation and transcription machinery confers ethanol tolerance in bacteria. *Proc Natl Acad Sci.* 2014 24;111 (25):E2576–85.
- [29] Block SS. Disinfection, sterilization, and preservation. Philadelphia, PA: Lippincott Williams & Wilkins; 2001.
- [30] Abuzaid A, Hamouda A, Amyes SGB. *Klebsiella pneumoniae* susceptibility to biocides and its association with *cepA*, *qacΔE* and *qacE* efflux pump genes and antibiotic resistance. *J Hosp Infect.* 2012 Jun;81 (2):87–91.
- [31] Vijayakumar R, Aboody M, AlFonaison M, et al. Determination of minimum inhibitory concentrations of common biocides to multidrug-resistant gram-negative bacteria. *Appl Med Res.* 2016;2(3):56.
- [32] Lambert RJW, Joynson J, Forbes B. The relationships and susceptibilities of some industrial, laboratory and clinical isolates of *Pseudomonas aeruginosa* to some antibiotics and biocides. *J Appl Microbiol.* 2001;91 (6):972–984.