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Transfer of plasmids harbouring *bla*$_{\text{OXA-48-like}}$ carbapenemase genes in biofilm-growing *Klebsiella pneumoniae*: Effect of biocide exposure

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ABSTRACT

The spread of OXA-48-encoding plasmids from *Klebsiella pneumoniae* (OXA-48-Kpn), especially successful high-risk (HR) clones, is a growing concern. Biofilm formation can contribute to the dissemination of OXA-48-Kpn. It is not known whether biocides can affect the transfer of OXA-48-Kpn in biofilm. The aim of this study was to evaluate the effect of biocides on the conjugation frequency (CF) of OXA-48-Kpn in both biofilm and planktonic cultures. For that, seven OXA-48-Kpn isolates (4 belonging to HR clones and 3 to non-HR clones) were selected as donors. Each isolate was mixed (1:1) with *Escherichia coli* J53 (recipient) and grown on polystyrene microplates without biocides (control) and with 0.25x MIC of triclosan (TRI), chlorhexidine digluconate (CHX), povidone-iodine (POV), sodium hypochlorite (SOD) or ethanol (ETH). The CF was calculated as the number of transconjugants/number of *E. coli* J53. The results showed that for isolates growing in the absence of biocide, the mean fold change in the CF in biofilm with respect to that determined in planktonic cells (CF-BF/CF-PK) was 0.2 in non-HR isolates and ranged from 2.0 to 14.7 in HR isolates. In HR isolates grown in the presence of biocide, especially CHX, TRI, and ETH, the fold changes in CF-BF/CF-PK decreased, whereas in non-HR isolates the fold changes were similar or increased slightly with CHX, ETH, SOD and POV. In conclusion, the fold changes in the CF-BF/CF-PK are higher in HR isolates comparing to non-HR isolates in abscence of biocides. The fold changes in CF-BF/CF-PK of the HR isolates in the presence of biocides varied with the type of biocides, whereas in non-HR isolates, biocides have no significant effect, or produce only a slight increase in the fold change of CF-BF/CF-PK.

1. Introduction

Plasmid-encoded *bla*$_{\text{OXA-48-like}}$ carbapenemases, which are spreading efficiently worldwide (Poirel et al., 2012), can have serious clinical and epidemiological consequences due to the limited treatment options available and the possibility of causing nosocomial outbreaks, particularly with isolates belonging to some of the successful high-risk (HR) clones of *K. pneumoniae* (Navon-Venezia et al., 2017). Bacterial biofilms not only provide a protective barrier against toxic compounds such as antibiotics, biocides, and heavy metals, but also create suitable environments for plasmid transfer (Otter et al., 2015). Bacterial biofilms are important in the pathogenesis of infection, facilitating the persistence of multidrug-resistant bacteria in the nosocomial environment and their spread from hidden reservoirs (Vergara-Lopez et al., 2013).

It has been shown that, apart from their bactericidal activity, sub-inhibitory concentrations of biocides can cause bacterial stress, inducing the SOS response and promoting the transfer of plasmids harbouring antibiotic resistance genes (Zhang et al., 2017). Even though OXA-48 is the most prevalent carbapenemase in many areas, there is no information about the effect of biocides on the transfer of plasmids encoding

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OXA-48 from biofilm-growing *K. pneumoniae*.

The aim of this study was to evaluate the effect of biofilm production and biocide exposure in the transfer of these plasmids in clinically relevant *K. pneumoniae* isolates using plasmids harbouring $bla_{\text{OXA-48-like}}$ carbapenemases. This information may help in designing optimal prevention and control strategies against nosocomial infections caused by *K. pneumoniae* producing plasmids encoding OXA-48-like carbapenemases.

2. Material and methods

2.1. Bacterial isolates

Seven *K. pneumoniae* clinical isolates belonging to different clonal types (STs) and with plasmids carrying $bla_{\text{OXA-48-like}}$ carbapenemase genes were selected as donors for this study (Table 1). The classification of isolates in HR or non-HR clones was performed according to the literature (Navon-Venezia et al., 2017). The plasmid incompatibility group was identified by PCR, using specific primers (Carattoli et al., 2015). *Escherichia coli J53* (azide-resistant) was used as recipient in plasmid conjugation assays. *K. pneumoniae* ATCC 700603 was used as control for biocide susceptibility.

2.2. Biocide susceptibility testing

The activities of triclosan (TRI; Sigma®), 2% chlorhexidine digluconate (CHX), povidone-iodine (POV; Betadine®), sodium hypochlorite (SOD; domestic bleach) and ethanol (ETH) against OXA-48-producing *K. pneumoniae* donors and *E. coli* J53 were tested by broth microdilution in Mueller–Hinton broth (MHB; Difco, Madrid, Spain) in a previous study (Gual-de-Torrella et al., 2021). The MICs (minimal inhibitory concentration) of each biocide, defined as the lowest concentration of biocide to prevent visible growth, were determined in triplicate.

2.3. Biofilm formation assay

Biofilm formation was determined using the violet crystal assay (Stepanović et al., 2000). Overnight cultures of *K. pneumoniae* carrying ***bla*-OXA-48** plasmids (donors) and *E. coli* J53 (recipient) were grown in Luria Bertani (LB) broth (Oxoid, Madrid) for 24 h at 37°. Donors and recipient were mixed in a 1:1 ratio. Five mL aliquots of each mixture were inoculated into four 6-well, flat-bottom polystyrene microplates (GreinerBioOne® International GmbH, North Carolina, USA) containing LB (control) or subinhibitory (0.25x MIC) concentrations of each biocide. The mixtures were covered with plastic lids and incubated for 24 h at 25 °C. Biofilms attached to microplates were washed five times with 200 µL of PBS buffer (90.1 %, 8.4 % Na2HPO4, 1.5 % KH2PO4). One of the 4 microplates containing attached bacteria (growing with or without biocides) was selected to determine biofilm production, whereas the remaining 3 plates were used to determine CF in biofilm. Attached bacteria were fixed with 99 % methanol (Fisher Scientific, Leicestershire, UK) for 15 min. Wells were filled with 200 µL of 2% crystal violet (VWR Chemicals, Barcelona, Spain) and left for 10 min. Plates were rinsed 3 times with sterile water. After air-drying the wells, 150 µL of 33 % glacial acetic acid (PanReac AppliChem, Barcelona, Spain) was added to each well. After 15 min of incubation, optical density (OD) at 595 nm was determined using an Infinite 200 PRO spectrophotometer (Tecan Trading AG®, Switzerland). The median OD of 3 independent determinations performed on 3 different days was used to quantify the amount of biofilm formed.

Table 1
Relevant characteristics of seven *K. pneumoniae* isolates harbouring IncL plasmids encoding OXA-48-like carbapenemase genes (Gual-de-Torrella et al., 2021).

Sequence type	High-riskclone	Plasmid-encoded genes		MIC (mg/L)[1]		
		OXA-48-type carbapenemase	CTX-M-type ESBL	ETP	IMP	MEM
ST11	Yes	$bla_{\text{OXA-245}}$	$bla_{\text{CTX-M-15}}$	**>1**	≤1	≤1
ST37	Yes	$bla_{\text{OXA-48}}$	No	**>1**	≤1	**4**
ST13	Yes	$bla_{\text{OXA-48}}$	No	**>1**	2	≤1
ST16	Yes	$bla_{\text{OXA-48}}$	$bla_{\text{CTX-M-15}}$	**>1**	≤1	≤1
ST437	No	$bla_{\text{OXA-245}}$	No	**>1**	≤1	≤1
ST846	No	$bla_{\text{OXA-48}}$	$bla_{\text{CTX-M-15}}$	**>1**	**4**	**>8**
ST899	No	$bla_{\text{OXA-48}}$	No	**>1**	2	2

[1] ETP: ertapenem, IMP: imipenem, MEM: meropenem. MICs associated with non-susceptibility appear in bold.

2.4. Transfer of plasmids encoding OXA-48-like carbapenemase genes

Plasmids encoding OXA-48-like carbapenemase genes were transferred by conjugation between donors and recipient growing in planktonic and biofilm cultures as described above. The unattached bacteria obtained from the 5 mL of each mixture of donor and *E. coli* J53 recipient (1:1) were considered planktonic-growing cells. The bacteria adhered to the 6 well flat-bottom polystyrene microplates were sonicated (JP Selecta™, Barcelona) for 5 min, and resuspended in the last 1 mL of PBS buffer (Tanner et al., 2017). This fraction of attached cells were considered biofilm-growing cells.

Serial dilutions (1:10) of 1 mL of both planktonic and biofilm cells were performed independently, and subcultured in chromogenic UTI medium agar (Oxoid, Spain) containing azide (10^5 mg/L) and ertapenem (0.125 mg/L) for the selection of transconjugants, and azide (10^5 mg/L) for the sele ction of *E. coli* J53. The conjugation frequency (CF) of plasmids encoding OXA-48-like carbapenemases was calculated as the number of colony-forming units (CFUs) of transconjugants selected with azide and ertapenem / number of CFUs *E. coli* J53 selected with azide medium. The average CF was determined from three independent experiments. Ten transconjugants from each biocide exposure condition (isolate and biocide) were selected to i) confirm the presence of $bla_{\text{OXA-48-like}}$ by PCR (Poirel et al., 2012), and ii) determinate the plasmid incompatibility group by PCR using specific primers (Carattoli et al., 2015), as described above.

2.5. Statistics

Statistical analysis was performed using IBM SPSS Statistics 18 (IBM Corporation, Armonk, NY). Differences of $p < 0.05$ were considered statistically significant, using the Student's *t*-test or the Mann–Whitney *U* test.

3. Results

E. coli J53 (recipient) showed MICs of biocides (mg/L) of 2.4 (CHX), 0.1 (TRI), 17.8 (ETH), 547 (SOD) and 3125 (POV), which were similar to those determined in a previous study against the OXA-48-producing *K. pneumoniae* isolates tested in this study (Gual-de-Torrella et al., 2021).

K. pneumoniae carrying $bla_{\text{OXA-48}}$ plasmids and belonging to HR clones showed a biofilm formation ability without exposure to biocides of 6 to 2 fold increase compared to non-HR clones, excluding ST437 isolate which showed the lowest capacity for biofilm formation (4 fold of decrease) compared to the other non-HR clones (Table 2).

Biofilm production in isolates belonging to HR clones that were exposed to CHX, TRI, ETH and SOD was not affected or was reduced by 20 %. In contrast, exposure to POV greatly affected biofilm production in these isolates, reducing its formation capacity between 80−40% (Fig. 1).

The effect of biocides on biofilm production in isolates belonging to

Table 2
Biofilm formation ability, expressed by the mean of the OD values of 3 independent determinations, in *K. pneumoniae* isolates carrying bla_{OXA-48} plasmids and belonging to high-risk clones or non-high risk clones, exposed or not (controls) to subinhibitory concentrations of biocides.

Biocide exposure[1]	Biofilm formation ability, expressed by the mean of the OD values in OXA-48-type-producing *K. pneumoniae* isolates belonging to							*E. coli* J53
	High-risk (HR) clones				non-HR clones			
	ST16 OXA-48	ST37 OXA-48	ST11 OXA-245	ST13 OXA-48	ST437 OXA-245	ST846 OXA-48	ST899 OXA-48	
NO (Control)	1.31 ± 0.11	0.86 ± 0.12	0.74 ± 0.15	1.11 ± 0.06	0.05 ± 0.02	0.22 ± 0.00	0.24 ± 0.02	0.30 ± 0.18
CHX	1.03 ± 0.18	0.95 ± 0.10	0.62 ± 0.18	1.01 ± 0.00	0.05 ± 0.05	0.21 ± 0.00	0.29 ± 0.05	0.36 ± 0.20
TRI	1.19 ± 0.20	0.89 ± 0.05	0.77 ± 0.16	0.98 ± 0.01	0.03 ± 0.01	0.19 ± 0.02	0.24 ± 0.26	0.27 ± 0.16
ETH	1.21 ± 0.26	0.99 ± 0.11	0.73 ± 0.17	1.12 ± 0.28	0.04 ± 0.01	0.25 ± 0.07	0.29 ± 0.37	0.34 ± 0.24
SOD	1.25 ± 0.21	0.92 ± 0.10	0.78 ± 0.03	1.26 ± 0.04	0.01 ± 0.00	0.20 ± 0.03	0.28 ± 0.03	0.21 ± 0.12
POV	0.36 ± 1.1	0.50 ± 0.16	0.27 ± 0.16	0.67 ± 0.13	0.07 ± 0.00	0.09 ± 0.03	0.26 ± 0.02	0.36 ± 0.24

[1] CHX: chlorhexidine digluconate, TRI: triclosan, ETH: ethanol, POV: povidone-iodine and SOD: sodium hypochlorite (domestic bleach). Biocide exposure was determined at a concentration equivalent to 0.25xMIC.

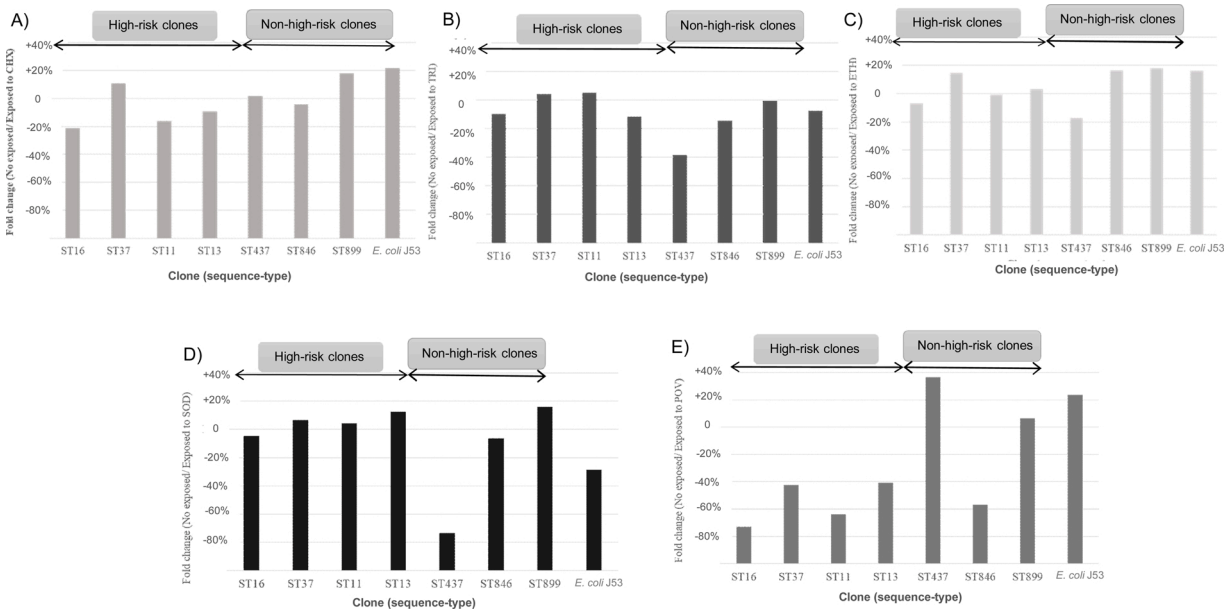

Fig. 1. Fold change in the biofilm production of high-risk and non-high-risk clones of *K. pneumoniae* carrying *bla*-$_{OXA-48}$ plasmids and *E. coli* J53 exposed or not (controls) to subinhibitory concentrations of chlorhexidine digluconate (CHX; panel A), triclosan (TRI; panel B), ethanol (ETH; panel C), sodium hypochlorite (SOD, domestic bleach; panel D) and povidone-iodine (POV, panel E).

non-HR clones was irregular and biocide dependent. Exposure to CHX only affected isolate belonging to clone ST899 and *E. coli* J53, which increased their biofilm formation capacity by 20 %. Exposure to TRI and SOD also had no effect on isolates belonging to non-HR clones, except for isolate belonging to clone ST437, and *E. coli* J53, that reduced its biofilm formation capacity by 40–80 % respectively. Exposure to ETH and POV increased biofilm formation capacity by 20–40 % in isolates belonging to most clones, except for isolate belonging to clone ST846, which considerably reduced (60 %) its biofilm formation on exposure to POV (Fig. 1).

The median of absolute CFs of the IncL plasmids harboring bla_{OXA-48} are shown in Table 3. In absence of biocide exposure the median CF ranged 4.4×10^{-6}–2.8×10^{-3} in planktonic cell and 1.2×10^{-6}–7.7×10^{-4} in biofilms. In presence of biocides, the CF in planktonic cells ranged 7.4×10^{-6}–2.4×10^{-3} (CHX), 5.2×10^{-5}–5.8×10^{-2} (TRI), 5.1×10^{-6}–4.9×10^{-3} (ETH), 8.4×10^{-6}-1.8×10^{-3} (SOD) and 1.2×10^{-5}–6.6×10^{-2} (POV). The CF in biofilms formed in presence of biocides reached values between 3.2×10^{-5}–6.9×10^{-4} (CHX), 6.8×10^{-6}–4.5×10^{-3} (TRI), 5.7×10^{-6}–2.1×10^{-3} (ETH), 6.7×10^{-6}–1.6×10^{-3} (SOD) and 3.2×10^{-6}–7.7×10^{-3} (POV) (Table 3).

As shown in Fig. 2, in the 4 HR isolates growing in the absence of biocides, the fold changes in the mean CF determined in biofilms with respect to planktonic cells (CF-BF/CF-PK) were variable, ranging from 2.0 (ST13) to 14.7 (ST16). By contrast, in the 3 non-HR isolates growing in the absence of biocides, the fold change in the CF-BF/CF-PK was much lower (0.2).

For HR isolates growing in the presence of the biocides CHX (ST11 > ST16 > ST13), TRI (ST37 > ST11 > ST16 > ST13), ETH (ST11 > ST37 > ST16), SOD (ST16), and POV (ST16) (see Fig. 2), there was a ≥2-fold decrease in CF-BF/CF-PK with respect to those determined in the absence of biocides (controls). The ST11 HR isolate was the only one in which a slight increase, rather than a decrease, was observed, which occurred with SOD (2-fold) POV (2.7-fold).

The fold changes in CF-BF/CF-PK of non-HR isolates growing in the presence of biocides with respect to those growing in the absence of biocides (controls) contrasted with those of HR isolates and were associated, in general, with a slight fold-change increase of 2–6 with CHX (ST899), ETH (ST899 > ST846), SOD (ST846 > ST899, ST437), and POV (ST437) (Fig. 1). The highest fold-change increase of 44 was observed for the non-HR isolate ST899 grown with ETH. TRI and POV were the only biocides in which a slight fold-change decrease (ST846) of 2 was observed.

Table 3
Absolute conjugation frequency of bla_{OXA-48} plasmids in planktonic (PK) and biofilms (BF) cells of *K. pneumoniae* isolates belonging to high-risk clones or non-high risk clones, exposed or not (controls) to subinhibitory concentrations of biocides.

Biocide exposure[1]	Growing condition[2]	Absolute conjugation frequency of blaOXA-48 plasmids of isolates belonging to						
		High-risk (HR) clones				non-HR clones		
		ST16 OXA-48	ST37 OXA-48	ST11 OXA-245	ST13 OXA-48	ST437 OXA-245	ST846 OXA-48	ST899 OXA-48
NO (Control)	PK	1.5×10^{-5}	4.4×10^{-6}	1.3×10^{-4}	2.0×10^{-5}	1.2×10^{-4}	2.8×10^{-3}	6.0×10^{-6}
	BF	2.2×10^{-4}	3.2×10^{-5}	7.7×10^{-4}	4.0×10^{-5}	2.7×10^{-5}	4.5×10^{-4}	1.2×10^{-6}
CHX	PK	5.6×10^{-5}	7.4×10^{-6}	8.7×10^{-5}	3.8×10^{-5}	1.7×10^{-4}	2.4×10^{-3}	1.7×10^{-4}
	BF	7.4×10^{-5}	3.2×10^{-5}	4.5×10^{-5}	3.7×10^{-5}	3.2×10^{-5}	6.9×10^{-4}	8.3×10^{-5}
TRI	PK	1.4×10^{-3}	5.3×10^{-4}	1.3×10^{-4}	8.2×10^{-5}	4.0×10^{-4}	5.8×10^{-2}	5.2×10^{-5}
	BF	8.5×10^{-4}	4.5×10^{-5}	6.8×10^{-6}	1.5×10^{-5}	2.0×10^{-4}	4.5×10^{-3}	4.8×10^{-5}
ETH	PK	4.3×10^{-5}	1.2×10^{-5}	7.8×10^{-5}	4.2×10^{-5}	4.4×10^{-5}	4.9×10^{-3}	5.1×10^{-6}
	BF	1.7×10^{-4}	1.5×10^{-5}	5.7×10^{-6}	6.6×10^{-5}	1.3×10^{-5}	2.1×10^{-3}	4.5×10^{-5}
SOD	PK	$7.3 \times 10{-}5$	8.4×10^{-6}	3.3×10^{-5}	7.1×10^{-4}	4.9×10^{-5}	1.8×10^{-3}	1.6×10^{-5}
	BF	1.1×10^{-4}	5.8×10^{-5}	3.9×10^{-4}	1.4×10^{-3}	2.0×10^{-5}	1.6×10^{-3}	6.7×10^{-6}
POV	PK	2.4×10^{-4}	1.7×10^{-5}	1.2×10^{-5}	2.7×10^{-5}	1.3×10^{-5}	6.6×10^{-2}	1.7×10^{-5}
	BF	6.7×10^{-4}	1.2×10^{-4}	1.9×10^{-4}	6.0×10^{-5}	1.4×10^{-5}	7.7×10^{-3}	3.2×10^{-6}

[1] CHX: chlorhexidine digluconate, TRI: triclosan, ETH: ethanol, POV: povidone-iodine and SOD: sodium hypochlorite (domestic bleach). Biocide exposure was determined at a concentration equivalent to 0.25xMIC.
[2] PK: planktonic growth, BF: biofilm growth.

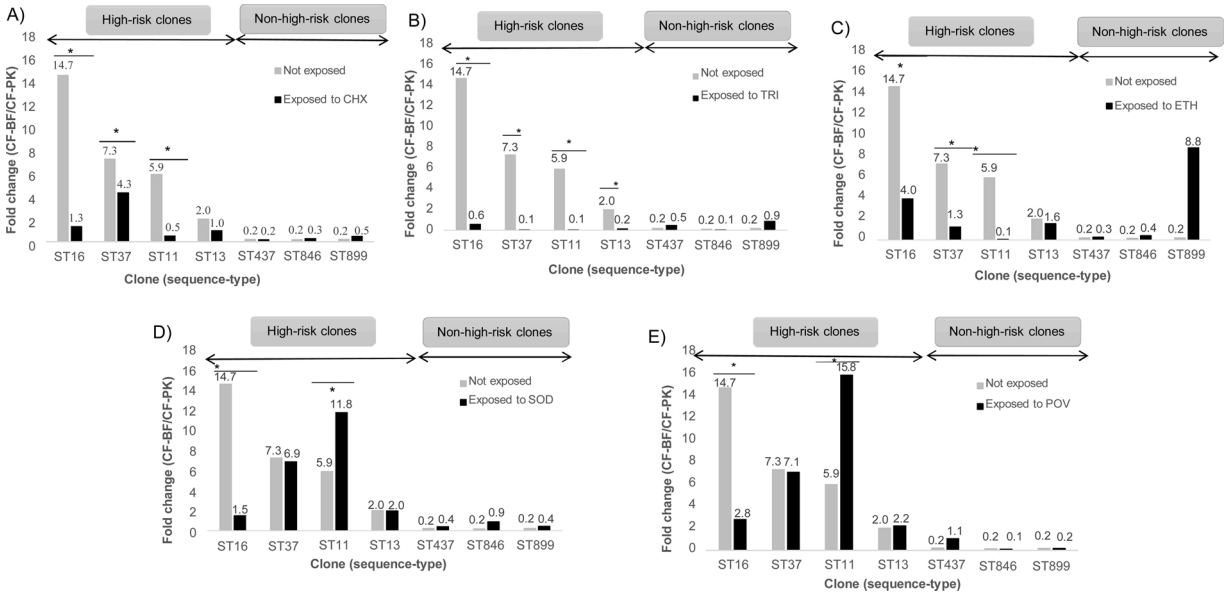

Fig. 2. Fold change in conjugation frequency in biofilm (CF-BF) of IncL-OXA-48- encoding plasmids and in planktonic cells (CF-PK) of isolates belonging to high-risk and non-high-risk clones, exposed or not (controls) to subinhibitory concentrations of chlorhexidine digluconate (CHX; panel A), triclosan (TRI; panel B), ethanol (ETH; panel C), sodium hypochlorite (SOD, domestic bleach; panel D) and povidone-iodine (POV, panel E) *p < 0.05.

4. Discussion

To our knowledge, this is the first time that the role of biofilm and the effect of biocide exposure on the horizontal transfer of plasmids encoding $bla_{OXA-48\text{-like}}$ carbapenemase genes in clinical isolates of *K. pneumoniae* has been investigated.

The ability of multidrug-resistant bacteria to form biofilm protects them from the action of antimicrobials, biocides, and host defence mechanisms. The presence of biofilms in environmental reservoirs facilitates patient colonisation, and thus nosocomial outbreaks (Vergara-Lopez et al., 2013). In a previous study performed with CTX-M-15-producing *K. pneumoniae,* it was demonstrated that the conjugation efficiency of the plasmid encoding CTX-M-15 was higher in biofilm (0.5/donor) than in planktonic cells (10^{-3}/donor) (Hennequin et al., 2012). Although there are several studies showing an strong association between successful plasmids harbouring antibiotics resistance genes and the belonging to a specific HR clone, both in gram positive and gram negative bacteria (Willems et al., 2011; Mathers et al., 2015; Dunn et al., 2019; David et al., 2020; Johnson, 2021), the information provided in relation to the CFs of these plasmids in HR clones of *K. pneumoniae* is limited. This led us to investigate whether the enhanced CF in biofilms also occurs in *K. pneumoniae* with plasmids encoding OXA-48-like, one of the most common carbapenemases.

In our study, for isolates that were grown without biocides, the biofilm formation ability and the fold changes in the CF-BF/CF-PK was higher in HR than in non-HR isolates. The cause of the enhanced plasmid transfer in ESBL- or carbapenemase-producing *K. pneumoniae* biofilms remains unknown, but it could give them an important survival advantage compared to non-HR isolates, contributing to their persistence and ability to spread in the hospital environment. This competitive advantage could be of particular importance in changing contaminated human and inorganic environments or reservoirs (e.g. medical devices such as flexible gastrointestinal endoscopes, hospital wastewater effluent and contaminated sinks), where bacterial populations usually

grow and form biofilms in response to adverse conditions (such as residual traces of biocides, antibiotics, or heavy metals) (Suleyman et al., 2018; Henly et al., 2019).

While many commercial biocides used as antiseptics and disinfectants in hospitals have been evaluated for their effectiveness against planktonic bacteria, there is less data available on the biocidal activity of these compounds against common nosocomial pathogens growing in biofilms (El-Azizi et al., 2016; Machuca et al., 2019) where they are generally more resistant to preservatives, disinfectants, and antiseptics than in planktonic growth (Scher et al., 2005). In our study, exposure to some biocides, such as CHX, TRI, ETH and SOD showed a moderate effect on biofilm formation, maintaining or reducing it slightly. Only exposure to POV was able to significantly decrease biofilm formation in isolates belonging to HR clones. Regarding the production of biofilm in the presence of biocides in isolates belonging non-HR clones, the effect was uneven, reducing biofilm formation in the presence of TRI or SOD in isolate belonging to clone ST437 or in the presence of POV in isolate belonging to clone ST846, although in general the biocides increased biofilm formation in isolates belonging to non-high risk clones.

The effect of biocides on frequency of conjugation, particularly in gram-negative bacteria, has received little attention, and the information available on Gram-positive bacteria is insufficient and refers to *Staphylococcus aureus*. . In our study, the fold changes in the CF-BF/CF-PK of the HR isolates in the presence of biocides varied with the type of biocides. As it was observed, the fold changes in CF-BF/CF-PK in HR isolates growing in the presence of biocides, particularly CHX, TRI, and ETH, were reduced with respect to those of HR isolates grown in the absence of biocides, which is consistent with the results of previous studies showing that the CF of plasmids, particularly in *S. aureus*, was reduced when growing in the presence of subinhibitory concentrations of certain biocides, such as cetrimide, CHX, POV, quaternary ammonium derivatives, and sodium dodecyl sulphate, among others (Pearce et al., 1999). In addition, our results suggest that CHX, TRI, and ETH could be useful to prevent or reduce the formation of biofilm and the transfer of $bla_{\text{OXA-48}}$-encoding plasmids, as well as the spread of $bla_{\text{OXA-48}}$-producing isolates belonging to these HR clones, as part of the prevention and control measures designed for nosocomial infections or outbreaks. With respect to the mechanisms that enhance plasmid transfer in biofilms, while the SOS response mediated by biocide-induced bacterial stress may have an important role to play in this negative effect of biocides, as has been demonstrated with some antibiotics, we still do not know which precise mechanism(s) is/are involved in the mobilization of plasmid-encoded $bla_{\text{OXA-48}}$ carbapenemases (Zhang et al., 2017; Beaber et al., 2004).

SOD and POV were observed to have a more variable effect on fold change in CF than was observed with CHX, TRI, and ETH, suggesting that SOD and POV could be useful to reduce the CF of the $bla_{\text{OXA-48}}$ plasmid-encoded carbapenemase in ST16 HR isolate, but not in the other three HR isolates. It is should also be highlighted that, in the ST11 HR isolate tested, there was a fold-change increase in CF-BF/CF-PK, instead of the reduction observed in the other HR isolates, associated with the exposure to SOD and POV. This could represent a potential competitive advantage for the ST11 HR isolate with respect to the other HR isolates tested under the selective pressure of SOD or POV, since it could efficiently co-transfer other genes related to resistance or tolerance to antibiotics, biocides, or heavy metals. This is of particular importance in contaminated hospital environments or reservoirs, since it may contribute to the maintenance or persistence of nosocomial outbreaks caused by $bla_{\text{OXA-48}}$-producing *K. pneumoniae* (Parkes et al., 2018; Cahill et al., 2019).

In non-HR isolates, and in contrast to the HR isolates, the fold changes in CF-BF/CF-PK determined in the presence of biocides versus those determined without biocides were associated, in general, with a slight increase in the CF. In the case of ETH and the non-HR ST899 isolate, the fold change in CF-BF/CF-PK was 44 times higher growing with ETH than without ETH, which is in line with the results reported by Seier-Petersen et al. (2014) on the conjugative transfer of Tn916 in *Bacillus subtilis*. The effect of ETH on ST899 could be related to a previous adaptation to growth in the presence of ETH, or to the induction of a pathway or mechanism unrelated to the SOS response. New studies are required to demonstrate this hypothesis.

All the OXA-48-producing *K. pneumoniae* transconjugants tested harbored the same plasmids carrying $bla_{\text{OXA-48}}$, belonging to the IncL incompatibility group, which is in agreement with the results of previous studies where the $bla_{\text{OXA-48}}$ gene is mainly encoded in IncL/M plasmid (Carattoli et al., 2015). “

This study was performed only with $bla_{\text{OXA-48}}$-encoding plasmids belonging to the IncL incompatibility group. Nevertheless, it would be interesting to broaden the study to plasmids encoding other carbapenemase genes, such as $bla_{\text{KPC-like}}$, $bla_{\text{NDM-like}}$ or $bla_{\text{VIM-like}}$, and to other relevant HR isolates (e.g. ST15, ST512).

These results could be of interest for the design of control programs suitable for multidrug-resistant bacteria, although the in vivo significance of these results is not yet known.

In conclusion, this study shows that biofilm production in *K. pneumoniae* producing IncL plasmid-encoded OXA-48-like carbapenemases is associated with a higher CF of these plasmids in HR isolates, especially ST16. Moreover, among the biocides tested, CHX, TRI and ETH produce a significant reduction in the CF of these plasmids in HR isolates, and, in general, a slight increase in CF in non-HR isolates.

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