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Impact of skin antisepsis on the prevalence of antibiotic-antiseptic cross-resistance genes in *Staphylococcaceae*

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Introduction

- **2 billion intravascular devices** (IVDs) are sold in the world annually (**300 million in the USA - 25 million in France**) (1). Occurrence of **infectious complications** mainly by ***Staphylococcus aureus* (17.3%)** and *Pseudomonas aeruginosa* (4.9%) (2).
- Recommendation of the French Society of Hospital Hygiene: use of an antiseptic solution prior to catheter insertion, with broad-spectrum biocidal activity (bacteria, viruses, fungi, etc.) (*ie.* **CHXOH : Chlorhexidine or PVIOH : Polyvidone iodine, both with OH : Alcohol; DAK : Sodium Hypochlorite** in the youngest)(3).
- However, the **association between antiseptic exposure and antibiotic resistance remains debated**, despite the **identification of potential cross-resistance genes** (4).

Objective

To evaluate in *Staphyloccocus* spp. isolated from colonized/infected IVDs the presence of antibiotic-antiseptic cross-resistance genes according to the antiseptic molecules used.

Methods

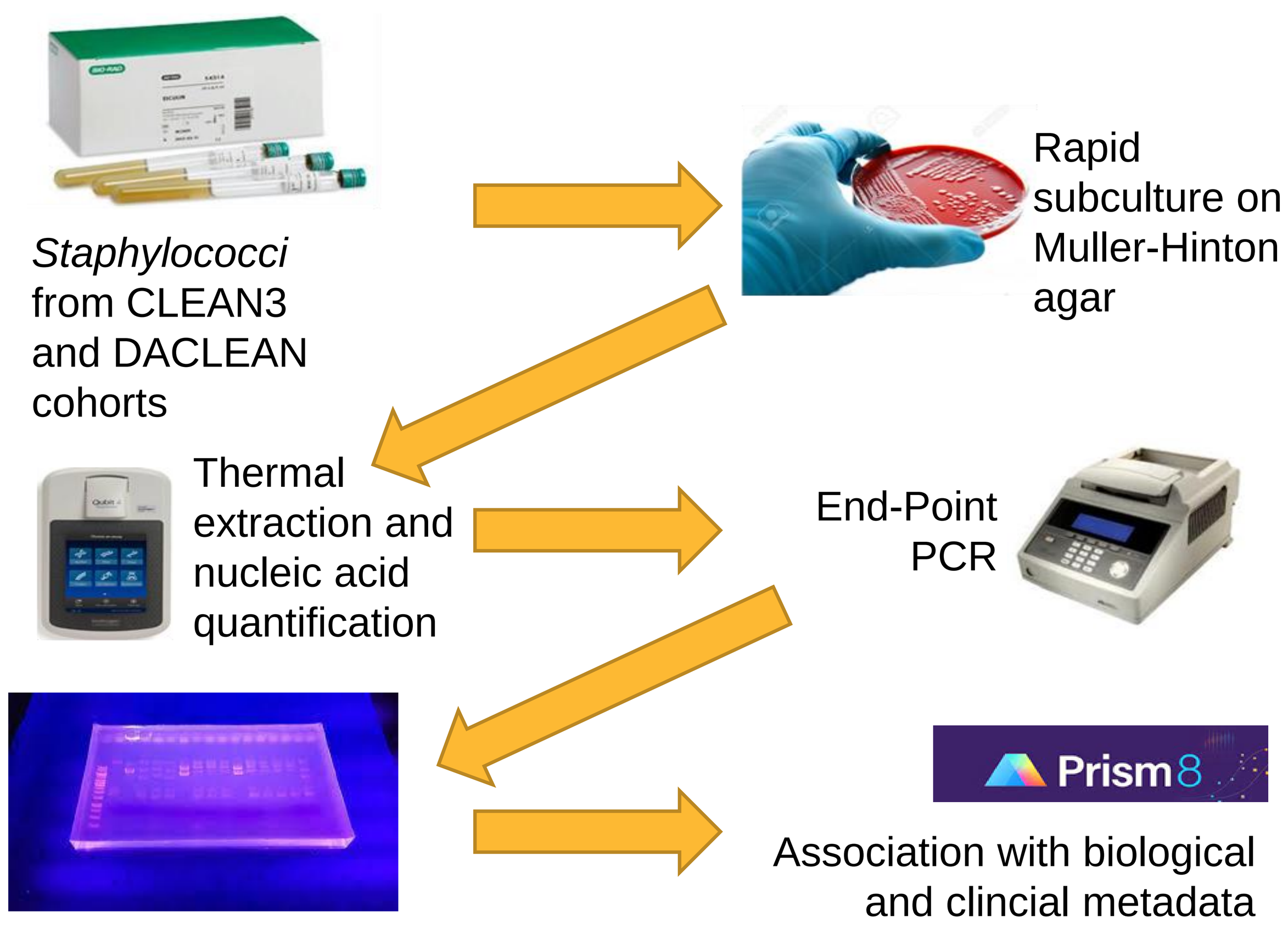

Figure 1. Analysis protocol. The strains studied (n=154) were derived from the CLEAN3 and DACLEAN clinical studies (5,6). *Staphylococci* were grown on blood agar before being extracted by heat shock (95°C 5 min). Extracts were assayed/diluted (100 ng per reaction) before amplification and separation of PCR products on agarose gel before statistical analysis according to the antiseptic used.

Gene	Primer[a]	Sequence (5'-3')	Annealing temperature (ºC)	Size (bp)
qacAB	qacAB_F	ATGCCTTATATTTATTTAATAATAGCC	42	321
	qacAB_R	ATGCGATGTTCCGAAAATGTTTAAC		
smr	smr_F	ATAAGTACTGAAGTTATTGGAAGT	48	285
	smr-R	TTCCGAAAATGTTTAACGAAACTA		
norA	norA_F	TTCACCAAGCCATCAAAAAG	45	620
	norA_R	CTTGCCTTTCTCCAGCAATA		
lmrS	lmrS_F	GCAAGCTTATGGCTAAAGTTGAATTAACAAC	53	1441
	lmrS_R	GCGGATCCTTAAAATTTCCTTCTATTACTTT		
mepA	mepA_F	ATGTTGCTGCTGCTCTGTTC	53	718
	mepA_R	TCAACTGTCAAACGATCACG		
sepA	sepA_F	GCAGTCGAGCATTTAATGGA	53	103
	sepA_R	ACGTTGTTGCAACTGTGTAAGA		

Table 1. Multiplexed PCR protocol. Amplifications were enabled by the primers published by Conceicao in two separate PCRs (7). Checks on agarose gels have been performed at the same time.

Results

	PVIOH (n=44)	CHXOH (n=45)	DAKALL (n=81)	DAK (n=42)	DAKOH (n=39)
Age (mean+/- SEM)	77.2 (2.7)	61.38 (2.3)	72.9 (1.8)	71.8 (2.4)	74.0 (2.5)
SexRatio (H/F)	30/14	31/14	51/29	28/14	24/15
Colonization rate (n; %)	32 (72.7)	25 (55.6)	49 (61.2)	23 (54.8)	27 (69.2)
Concentration (log10 mean; sd)	3.34 (1.2)	2.62 (1.1)	2.59 (1.0)	2.58 (1.1)	2.95 (1.2)
Bacteria					
S. aureus (n=16; 9.5%)	3 (6.8)	4 (8.9)	9 (11.1)	5 (11.9)	4 (10.3)
S. epidermidis (n=100; 59.2%)	30 (68.2)	30 (66.7)	40 (49.4)	18 (42.9)	22 (56.4)
S. hominis (n=21; 12.4%)	4 (9.1)	2 (4.4)	15 (18.5)	11 (26.2)	4 (10.3)
S. capitis (n=14; 8.2%)	2 (4.5)	4 (8.89)	9 (11.1)	5 (11.9)	4 (10.3)
S. haemolyticus (n=11; 6.5%)	3 (6.8)	4 (8.89)	4 (4.9)	2 (4.8)	2 (5.1)
S. pettenkoferi (n=2; 1.2%)	0 (-)	0 (-)	2 (2.5)	0 (-)	2 (5.1)
S. warneri (n=2; 1.2%)	1 (2.3)	0 (-)	1 (1.2)	0 (-)	1 (2.6)
S. lugdunensis (n=1; 0.6%)	1 (2.3)	0 (-)	0 (-)	0 (-)	0 (-)
S. succinus (n=1; 0.6%)	0 (-)	0 (-)	1 (1.2)	1 (2.4)	0 (-)
S. simulans(n=1; 0.6%)	0 (-)	1 (-)	0 (-)	0 (-)	0 (-)

Table 2. Distribution of the analyzed strains according to the antiseptic procedure.

Gene (n;%)	PVIOH (n=44)	CHXOH (n=45)	DAKALL (n=81)	DAK (n=42)	DAKOH (n=39)
qacA/B (n=81; 47.6%)	22 (50.0)	7 (15.6)	51 (63.8)	29 (70.7)	22 (56.4)
lmrS (n=57; 33.5%)	12 (27.3)	5 (11.1)	39 (48.8)	20 (48.8)	19 (48.7)
norA (n=84; 49.4%)	20 (45.5)	34 (75.6)	30 (37.5)	15 (36.6)	15 (38.5)
mepA (n=51; 30%)	25 (56.8)	5 (11.1)	23 (28.8)	13 (31.7)	8 (20.5)
sepA (n=45; 26.5%)	8 (18.2)	11 (24.4)	24 (30.0)	16 (39.0)	10 (25.6)
smr (n=27; 15.9%)	11 (25.0)	8 (17.8)	8 (10.0)	3 (7.32)	5 (12.8)
Number of gene per bacteria (mean; SEM)	2.23 (0.2)	1.56 (0.2)	2.21 (0.1)	2.37 (0.2)	2.03 (0.2)

p-value			
DAK/DAKOH	DAKOH/PVIOH	DAK/PVIOH	DAKALL/PVIOH
0.2	0.5	0.6	0.9
DAK/CHG	DAKOH/CHG	PVIOH/CHG	DAKALL/CHG
<0.01	0.1	<0.01	<0.01

Table 3. Comparison of cross-resistance gene prevalence according to the antisepsis used. Strains isolated after selection by CHG have fewer cross-resistance genes. (p<0,05).

norA (n=84)	Pos.	Neg.
DAKALL	30 (37.5)	50 (62.5)
DAK	15 (36.6)	26 (63.4)
DAKOH	15 (38.5)	24 (61.5)
PVIOH	20 (45.5)	24 (54.5)
CHXOH	34 (75.6)	11 (24.4)

p-value	DAK/DAKOH	DAKOH/PVIOH	DAK/PVIOH	DAKALL/PVIOH	DAK/CHXOH	DAKOH/CHXOH	PVIOH/CHXOH	DAKALL/CHXOH
norA	1	0.66	0.51	0.45	<0.01	<0.01	<0.05	<0.001

smr (n=27)	Pos.	Neg.
DAKALL	8 (10.0)	72 (90.0)
DAK	3 (7.3)	38 (92.7)
DAKOH	5 (12.8)	34 (87.2)
PVIOH	11 (25.0)	33 (75.0)
CHXOH	8 (17.8)	37 (82.2)

p-value	DAK/DAKOH	DAKOH/PVIOH	DAK/PVIOH	DAKALL/PVIOH	DAK/CHXOH	DAKOH/CHXOH	PVIOH/CHXOH	DAKALL/CHXOH
smr	0.48	0.18	<0.05	<0.05	0.2	0.56	0.45	0.27

sepA (n=45)	Pos.	Neg.
DAKALL	24 (30.0)	56 (70.0)
DAK	16 (39.0)	25 (61.0)
DAKOH	10 (25.6)	29 (74.4)
PVIOH	8 (18.2)	36 (81.8)
CHXOH	11 (24.4)	34 (75.6)

p-value	DAK/DAKOH	DAKOH/PVIOH	DAK/PVIOH	DAKALL/PVIOH	DAK/CHXOH	DAKOH/CHXOH	PVIOH/CHXOH	DAKALL/CHXOH
sepA	0.24	0.44	0.05	0.2	0.17	1	0.61	0.54

Table 4. Antiseptic-antibiotic cross-resistance genes vary according to the antiseptic used. Bacteria selected by the CHG antiseptic protocol are significantly less frequent carriers of resistance genes (p<0.05) compared to other antiseptics. *sepA* is statistically associated with *qacA/B*, smr and *mepA* (p<0.05). *norA* tends to be associated with *lmrS* (p<0.1).

		mepA Pos.	*mepA* Neg.	p-value
sepA	Pos.	18	25	0.1
	Neg.	35	91	

		norA Pos.	*norA* Neg.	p-value
lmrS	Pos.	22	34	0.1
	Neg.	62	51	

		smr Pos.	*smr* Neg.	p-value
sepA	Pos.	11	32	<0.05
	Neg.	15	110	

		norA+qacA/B Pos.	*norA+qacA/B* Neg.	p-value
sepA	Pos.	14	7	<0.05
	Neg.	22	34	

		qacA/B Pos.	*qacA/B* Neg.	p-value
sepA	Pos.	29	14	<0.01
	Neg.	51	75	

		mepA+qacA/B Pos.	*mepA+qacA/B* Neg.	p-value
sepa	Pos.	16	24	<0.05
	Neg.	14	54	

Table 5. Association of the different resistance genes with each other. *sepA* is statistically associated with *qacA/B*, smr and *mepA* (p<0.05). *norA* tends to be associated with *lmrS* (p<0.1).

	Absence colonization (n=63)	Presence Colonization (n=106)	p-value
Number of gene per bacteria (mean; SEM)	2.2 (0.2)	2.0 (0.1)	0.3
Gene name (n)			
norA (n=84)	29 (46.0)	55 (51.9)	0.5
mepA (n=53)	23 (36.5)	30 (28.3)	0.3
qacA/B (n=80)	30 (47.6)	50 (47.2)	1
sepA (n=43)	21 (33.3)	22 (20.8)	0.1
lmrS (n=56)	23 (36.5)	33 (31.1)	0.5
smr (n=27)	11 (17.5)	16 (15.1)	0.7
Gene association (n;%)			
mepA+qacA/B (n=30)	14 (22.2)	16 (15.1)	0.3
qacA/B+sepA (n=29)	15 (23.8)	14 (13.2)	0.1

Table 6. Association of resistance genes with colonization of intra-vascular devices. A trend is found between low bacterial concentration and *sepA* or the association *qacA/B+sepA* (p<0.1).

	S.aureus (n=16)	CNS (n=153)	pvalue
Number of gene per bacteria (mean; SEM)	3.4 (0.2)	1.9 (0.1)	<0.001
Gene name (n)			
norA (n=84)	10 (62.5)	70 (45.8)	0.3
mepA (n=53)	9 (56.3)	44 (28.8)	<0.05
qacA/B (n=80)	10 (62.5)	70 (45.8)	0.3
sepA (n=43)	7 (43.8)	36 (23.5)	0.1
lmrS (n=56)	15 (93.8)	41 (26.8)	<0.001
smr (n=27)	3 (11.1)	24 (15.7)	0.7
Gene association (n;%)			
mepA+qacA/B (n=30)	5 (31.3)	25 (16.3)	0.2
qacA/B+sepA (n=29)	7 (43.8)	22 (14.4)	<0.001

Table 7. Difference in prevalence of cross-resistance genes according to *Staphylococceae* species. *lmrS, mepA* and the *qacA/B+sepA* combination are significantly more frequent in *S. aureus* (p<0.05).

Betalactam	Oxacilline S (n=101)	Oxacilline R (n=52)	p-value
Number of gene per bacteria (mean; SEM)	1.9 (0.1)	2.1 (0.2)	0.3
Gene name (n)			
norA (n=76)	50 (49.5)	26 (50.0)	1
mepA (n=44)	30 (29.7)	14 (26.9)	0.9
qacA/B (n=71)	43 (42.6)	28 (53.9)	0.1
sepA (n=35)	18 (17.8)	17 (32.7)	<0.05
lmrS (n=48)	37 (36.6)	11 (21.2)	0.5
smr (n=25)	11 (10.9)	14 (26.9)	<0.05

Quinolon	Wild-type (n=109)	Resistant (n=32)	p-value
Number of gene per bacteria (mean; SEM)	2.0 (0.1)	1.9 (0.2)	0,6
Gene name (n)			
norA (n=67)	54 (49.5)	13 (40.6)	0,4
mepA (n=44)	36 (33.0)	13 (40.6)	0,5
qacA/B (n=68)	52 (47.7)	16 (50.0)	0,8
sepA (n=32)	20 (18.4)	12 (37.5)	<0.05
lmrS (n=47)	42 (38.5)	5 (15.6)	<0.05
smr (n=22)	15 (13.8)	7 (21.8)	0,3

Aminoglycosides	Wild-type (n=117)	Resistant (n=36)	p-value
Number of gene per bacteria (mean; SEM)	1.9 (0.1)	2.2 (0.2)	0,3
Gene name (n)			
norA (n=77)	58 (49.57)	19 (52.8)	0,9
mepA (n=44)	36 (30.8)	8 (22.2)	0,4
qacA/B (n=71)	52 (44.4)	19 (52.8)	0,5
sepA (n=35)	20 (17.1)	15 (41.7)	<0.01
lmrS (n=48)	42 (35.9)	6 (16.7)	<0.05
smr (n=25)	14 (12.0)	11 (30.6)	<0.05

Rifampicin	Susceptible (n=144)	Resistant (n=9)	p-value
Number of gene per bacteria (mean; SEM)	2.0 (0.1)	1.1 (0.4)	<0.05
Gene name (n)			
norA (n=77)	73 (50.7)	4 (44.4)	0,8
mepA (n=45)	44 (30.6)	1 (11.1)	0,3
qacA/B (n=71)	70 (48.6)	1 (11.1)	<0.05
sepA (n=34)	33 (22.9)	1 (11.1)	0,7
lmrS (n=48)	46 (31.9)	2 (22.2)	0,7
smr (n=25)	24 (16.7)	1 (11.1)	1

Table 8. Association of different resistance genes with antibiotic resistance phenotypes. Resistance to β-lactams, quinolones, aminoglycosides and rifampicin varies with the genes found (p<0.05) with the exception of *norA* and *mepA* (p>0.05)

Oxacilline R	Pos.	Neg.
DAKALL	17 (24.6)	52 (75.4)
DAK	10 (29.4)	24 (70.6)
DAKOH	7 (20.0)	28 (80.0)
PVIOH	12 (30.0)	28 (70.0)
CHXOH	23 (52.3)	21 (47.7)

Aminoglycoside R	Pos.	Neg.
DAKALL	12 (17.4)	57 (82.6)
DAK	6 (17.6)	28 (82.4)
DAKOH	6 (17.1)	29 (82.9)
PVIOH	5 (12.5)	35 (87.5)
CHXOH	19 (42.2)	26 (57.8)

Quinolone R	Pos.	Neg.
DAKALL	10 (14.5)	59 (85.5)
DAK	3 (8.8)	31 (91.2)
DAKOH	7 (20.0)	28 (80.0)
PVIOH	7 (17.5)	33 (82.5)
CHXOH	15 (46.9)	17 (53.1)

Rifampicin R	Pos.	Neg.
DAKALL	2 (2.9)	67 (97.1)
DAK	1 (2.9)	33 (97.1)
DAKOH	1 (2.9	34 (97.1)
PVIOH	0 (0)	40 (100)
CHXOH	7 (15.9)	37 (84.1)

p-value	DAK/DAKOH	DAKOH/PVIOH	DAK/PVIOH	DAKALL/PVIOH	DAK/CHXOH	DAKOH/CHXOH	PVIOH/CHXOH	DAKALL/CHXOH
Oxacilline R	0.41	0.43	1	0.65	0.06	<0.01	<0.05	<0.01
Aminoglycoside R	1	0.75	0.74	0.59	<0.05	<0.05	<0.01	<0.01
Quinolone R	0.31	1	0.33	0.79	<0.001	<0.05	<0.01	<0.01
Rifampicin R	1	0.47	0.46	0.53	0.13	0.07	<0.05	<0.05

Table 9. Antibiotic resistance of strains isolated after antisepsis differs depending on the molecule used. The use of CHX is more frequently associated with resistance to β-lactams, quinolones, aminoglycosides and rifampicin, than other antiseptic molecules (p<0.05).

Discussion

Antibiotic resistance is different between clinical strains isolated after exposure to antiseptic molecules

In contrast to phenotypic antibiotic resistance, the results suggest that **CHX selects fewer strains with cross-resistance genes than other antiseptics**.

Conclusion

Larger studies are needed to evaluate the impact of antiseptic procedure on other strains of clinical interest, both Gram-positive and Gram-negative, such as *Enterobacterales* and *Pseudomonales*.

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