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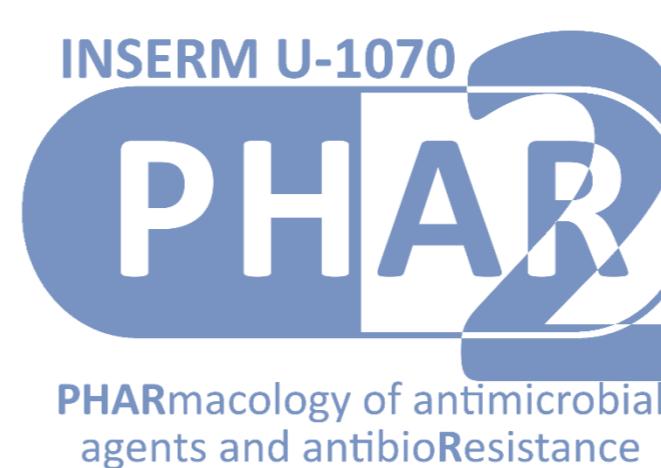
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Impact of skin antisepsis on the prevalence of antibiotic-antiseptic cross-resistance genes in *Staphylococcaceae*

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

Methods

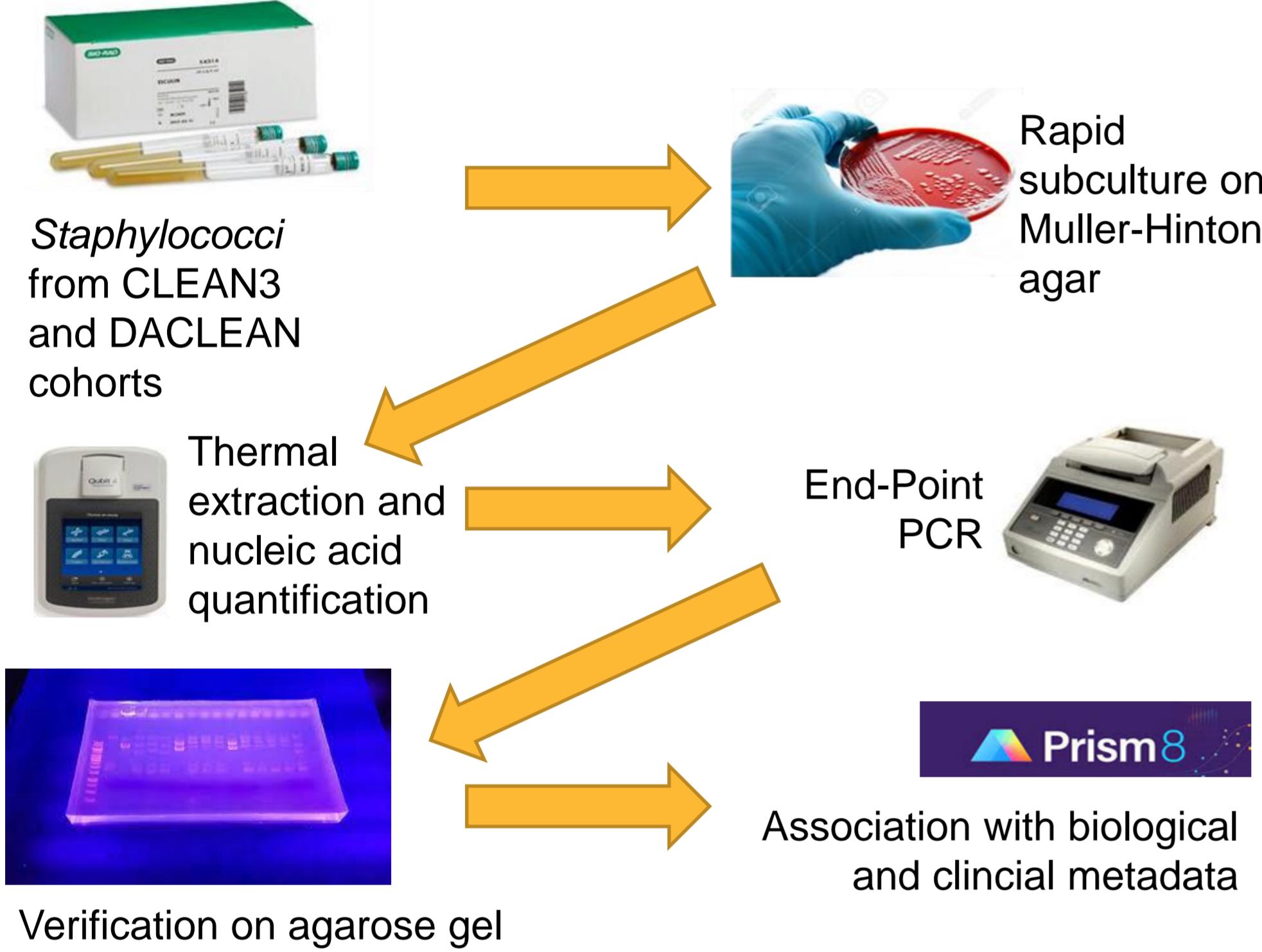


Figure 1. Analysis protocol. The strains studied (n=154) were derived from the CLEAN3 and DACLEAN clinical studies (5,6). *Staphylococci* were grown on blood agar before being extracted by heat shock (95°C 5 min). Extracts were assayed/diluted (100 ng per reaction) before amplification and separation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

Gene	Primer ^a	Sequence (5'-3')	Annealing temperature (°C)	Size (bp)
qacAB	qacAB_F	ATGCCTTATTTTATAATAATAGCC	42	321
	qacAB_R	ATGGATGTTCCGAAATGTIIAAC		
smr	smr_F	ATAAGTACTGAAGTTATTGAACT	48	285
	smr_R	TTCCGAAAATGTTAACGAAACTA		
norA	norA_F	TTCACCAAGGCCATCAAAG	45	620
	norA_R	CTTGCTTTCTCCAGCAATA		
lmrS	lmrS_F	GCAAGCTTATGGCTAAAGTGAATTAAACAC	53	1441
	lmrS_R	GGCGATCCTAAATTCTCTTCTATTACTTT		
mepA	mepA_F	ATGTTGCTGCTGCTGTTC	53	718
	mepA_R	TCAACTGTCAAAGCATCAG		
sepA	sepA_F	GCAGTCGAGCATTAATGG	53	103
	sepA_R	ACGTTGTCGAATGTGTAAGA		

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.

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 *Enterobacteriales* and *Pseudomonales*.

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	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					
<i>S. aureus</i> (n=16; 9.5%)	3 (6.8)	4 (8.9)	9 (11.1)	5 (11.9)	4 (10.3)
<i>S. epidermidis</i> (n=100; 59.2%)	30 (68.2)	30 (66.7)	40 (49.4)	18 (42.9)	22 (56.4)
<i>S. hominis</i> (n=21; 12.4%)	4 (9.1)	2 (4.4)	15 (18.5)	11 (26.2)	4 (10.3)
<i>S. capitis</i> (n=14; 8.2%)	2 (4.5)	4 (8.89)	9 (11.1)	5 (11.9)	4 (10.3)
<i>S. haemolyticus</i> (n=11; 6.5%)	3 (6.8)	4 (8.89)	4 (4.9)	2 (4.8)	2 (5.1)
<i>S. pettenkoferi</i> (n=2; 1.2%)	0 (-)	0 (-)	2 (2.5)	0 (-)	2 (5.1)
<i>S. warneri</i> (n=2; 1.2%)	1 (2.3)	0 (-)	1 (1.2)	0 (-)	1 (2.6)
<i>S. lugdunensis</i> (n=1; 0.6%)	1 (2.3)	0 (-)	0 (-)	0 (-)	0 (-)
<i>S. succinus</i> (n=1; 0.6%)	0 (-)	0 (-)	1 (1.2)	1 (2.4)	0 (-)
<i>S. simulans</i> (n=1; 0.6%)	0 (-)	1 (-)	0 (-)	0 (-)	0 (-)

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

		p-value								
norA (n=84)	Pos.	Neg.	DAK/DAKOH	DAKOH/PVIOH	DAK/PVIOH	DAKALL/PVIOH	DAK/CHXOH	DAKOH/CHXOH	PVIOH/CHXOH	DAKALL/CHXOH
DAKALL	30 (37.5)	50 (62.5)								
DAK	15 (36.6)	26 (63.4)	1							
DAKOH	15 (38.5)	24 (61.5)		0.66	0.51	0.45	<0.01	<0.01	<0.05	<0.001
PVIOH	20 (45.5)	24 (54.5)								
CHXOH	34 (75.6)	11 (24.4)								
smr (n=27)	Pos.	Neg.	DAK/DAKOH	DAKOH/PVIOH	DAK/PVIOH	DAKALL/PVIOH	DAK/CHXOH	DAKOH/CHXOH	PVIOH/CHXOH	DAKALL/CHXOH
DAKALL	8 (10.0)	72 (90.0)								
DAK	3 (17.3)	38 (92.7)	0.48							
DAKOH	5 (12.8)	36 (87.2)		0.18	<0.05	<0.05				
PVIOH	11 (25.0)	33 (75.0)								
CHXOH	8 (17.8)	37 (82.2)								
sepA (n=45)	Pos.	Neg.	DAK/DAKOH	DAKOH/PVIOH	DAK/PVIOH	DAKALL/PVIOH	DAK/CHXOH	DAKOH/CHXOH	PVIOH/CHXOH	DAKALL/CHXOH
DAKALL	24 (30.0)	56 (70.0)								
DAK	16 (39.0)	25 (61.0)	0.24							
DAKOH	10 (25.6)	29 (74.4)		0.44	0.05	0.2				
PVIOH	8 (18.2)	36 (81.8)								
CHXOH	11 (24.4)	34 (75.6)								

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).

		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).

		Wild-type (n=101)	Resistant (n=52)	p-value
Number of gene per bacteria (mean; SEM)		1.9 (0.1)	2.1 (0.2)	0.3
Gene name (n)</				