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Infections of implantable cardiac devices by biofilm forming bacteria in western Algeria hospitals

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Abstract:

Background: The significant increase in the use of implantable cardiac devices (ICDs) has been accompanied by biofilm formation and increase rate of infection on the devices. The purpose of our study is to describe the clinical and microbiological findings of infection of ICDs in the cardiology units of western Algeria hospitals.
Methodology: All patients with clinical diagnosis of ICD infections or infective endocarditis upon removal of their ICDs from December 2012 to August 2014 in cardiology units of 4 Algerian hospitals were included in the study. Each element of the ICD pocket and lead was separately sonicated in sterile saline, inoculated onto Chapman and MacConkey agar plates and incubated aerobically at 37°C for colony count after 24 hours. Biochemical identification of the bacteria isolates was made by API 20E, API 20 NE and API Staph, and confirmed by Siemens Healthcare Diagnostics WalkAway® 96 Plus System. Antibiotic susceptibility testing on each isolate was performed by the disk diffusion method on Mueller Hinton agar. Biofilm formation was detected by Congo Red Agar (CRA) and Tissue Culture Plate (TCP) methods, and hydrophobicity of the bacterial cell was determined by the MATH protocol.
Results: Over a period of twenty-one months, 17 ICDs were removed from patients with post-operative infections; 6 (35.3%) had early infection of ICD and 11 (64.7%) had late ICD infection. Fifty-four bacterial strains were isolated and identified, with coagulase-negative staphylococci being the predominant bacteria with 46.3% (25/54). There was no significant association between hydrophobicity and antimicrobial resistance in the 54 isolates but there is positive correlation between biofilm production and antimicrobial resistance, with the strongest biofilm producers resistant to more than one antibiotic. Four independent predictors of infection of resynchronization devices were reported; reoperation, multi-morbidity, long procedure, and ICD implantation.
Conclusion: Our study is the first in Algeria to describe microbiological characteristics of ICD infection. The bacteria in the biofilm were protected, more resistant and tolerated high concentrations of antibiotics and thus played a major role in the development of ICD infections. Despite the improvements in ICD design and implantation techniques, ICD infection remains a serious challenge.

Keywords: implantable cardiac devices, staphylococci, resistance, biofilm, hydrophobicity

Infections des dispositifs cardiaques implantables par des bactéries formant un biofilm dans les hôpitaux de l'ouest Algérien

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Résumé:

Contexte: L'augmentation significative de l'utilisation des dispositifs cardiaques implantables est un risque majeur d'augmentation du taux d'infection et donc du risque de formation d'un biofilm sur ce genre de dispositifs. L'objectif de notre étude est de décrire les résultats cliniques et microbiologiques de l'infection sur les dispositifs cardiaques implantables (DCI) dans les unités de cardiologie des hôpitaux de l'ouest Algérien.
Méthodologie: Tous les patients cliniquement diagnostiqués avec une infection sur DCI, ou une endocardite infectieuse et ayant subit un retrait de leur dispositif cardiaque sont inclus dans cette étude et cela sur une période entre décembre 2012 et aout 2014 dans 4 unités de cardiologie. Chaque élément du DCI (boitier et sonde) est trempé séparément dans une solution saline stérile, ensemencé sur deux milieux de culture, un milieu de Chapman et un milieu MacConkey et incubé en aérobiose à 37°C pour la numération des colonies après 24 heures. L'identification biochimique des isolats de bactéries est effectuée par le API 20E, API 20 NE et API Staph, et confirmée par le système WalkAway® 96 Plus de Siemens Healthcare Diagnostics. Les tests de sensibilité aux antibiotiques de chaque isolat sont effectués par la méthode de diffusion des disques sur gélose de Mueller Hinton. La formation d'un biofilm est détectée par les méthodes de la gélose rouge du Congo (CRA) et de la plaque de culture tissulaire (TCP), et l'hydrophobicité de la cellule bactérienne est déterminée par le protocole MATH.
Résultats: Sur une période de 21 mois, 17 DCI sont retirés de patients atteints d'infections postopératoires; 6 patients (35,3%) sont identifiés comme ayant une infection précoce sur leurs DCI et 11 patients (64,7%) ayant une infection tardive. Cinquante-quatre souches bactériennes sont isolées et identifiées, les staphylocoques à coagulase négative étant les bactéries prédominantes avec 46,3% (25/54). Il n'y a pas d'association significative entre l'hydrophobicité et la résistance aux antimicrobiens dans les 54 isolats, mais il existe une corrélation positive entre la production de biofilm et la résistance aux antimicrobiens, les plus puissants en biofilm sont résistant à plus d'un antibiotique. Quatre facteurs prédictifs indépendants d'infection des dispositifs cardiaques implantable sont retrouvés dans ce travail: ré-intervention, longue procédure, sujets multi-tarés, et implantation d'un DCI
Conclusion: Notre étude est la première en Algérie à décrire les caractéristiques microbiologiques de l'infection des DCI. Les bactéries présentes dans le biofilm sont protégées, plus résistantes et tolèrent de fortes concentrations d'antibiotiques et jouent ainsi un rôle majeur dans le développement des infections par DCI. Malgré des améliorations dans les techniques de conception et d'implantation de DCI, l'infection des dispositifs cardiaques implantables reste un problème grave et très couteux.

Mots-clés: dispositifs cardiaques implantables; staphylocoque; résistance; biofilm; hydrophobicité

Introduction:

The permanent implantable cardiac device (ICD) is one of the most widely used cardiac rhythm control devices. Since their introduction in the early 1980s, ICD have become a life-saving therapeutic tool for patients with ventricular arrhythmia. Virtually all the countries surveyed showed significant rise in the use of ICD, the largest implanter being the USA with 434 new implants per million populations (1). Despite improvements in cardiac device design, application of timely infection control practices, and administration of antibiotic prophylaxis at the time of device placement, infections continue to be observed. The infection rate is highly variable, ranging from 0.5 to 12% (2,3).

Infection of implantable cardiac device (ICD) may be local, limited to the pulse generator pocket and/or the sub-cutaneous portion of the leads, or systemic, involving the transvenous intravascular electrode (4). Several factors have been reported to be associated with a greater risk of ICD infections and these include; immuno-suppression (e.g. renal dysfunction and corti-

costeroid use); oral anticoagulation use; co-existing illnesses; periprocedural factors including failure to administer perioperative anti-microbial prophylaxis; device revision/replacement; and operators' experience (5).

Because of the invasiveness, these devices have multiple comorbidities in ICD recipients, the benefits of which can be overshadowed by infectious complications. Infection of ICD is usually a serious and fearsome complication requiring both complete removal of the infected device and systemic antimicrobial treatment (5,6). Although the implantation of pacemakers is a procedure characterized by low rate of complication, infections, mainly of the pace-maker generator pocket, is one of the most common complications with incidence ranging from 1 to 5%. Despite the low incidence, such infections have a worrying development, mostly because it is associated with high morbidity and potential fatality (7).

Microbiological diagnosis of ICD infection is of great importance for appropriate treatment. Staphylococcal species including *Staphylococcus aureus* and coagulase negative staphylococci (CoNS) represent the

majority of bacterial agents of ICD infection (8,9). However, some unusual microorganisms such as *Propionibacterium* spp., *Corynebacterium* spp., *Acinetobacter baumannii*, and *Haemophilus influenzae* may also be involved and antibiotic resistance is often detected in them (10,11). Furthermore, one major concern among professionals and bacteriologists is the risk of biofilm formation on the ICDs which consequently favors infection. In recent decades, biofilm formations have been widely reported to be responsible for nosocomial infections especially of prosthetic implants, tubes, leads and catheters (12,13).

It is speculated that the presence of an ICD is conducive for microbial colonization contributing greatly to the development of biofilms, which in turn, explains the occurrence of endocarditis (14,15). It is interesting to note that biofilm consists of a structured medium of microbial cells that adhere to a solid surface and is surrounded by a matrix of extracellular polymeric substances. This microbial combination is a form of protection encouraging symbiotic relationships, tolerance, as well as antimicrobial resistance (16).

The goal of this study is to first describe the bacteriological characteristics of ICD infections over a period of 2 years in four cardiology units of one private and three university hospital centers in western Algeria, and secondly to determine whether the isolated bacteria have the ability to form biofilms that may be responsible for triggering infection, especially in late stage endocarditis.

Materials and method:

Study setting:

This study is carried out at the Laboratory of Microbiology Applied to Food, Biomedical and Environment (LAMAABE) of the University Abou-Bekr Bekaid-Tlemcen, Algeria.

Subjects

All the patients with clinical diagnosis of ICD infection or infective endocarditis upon removal of their cardiac devices from December 2012 to August 2014 in four cardiology units of three hospitals in Algeria were included in this study. The clinical diagnosis of ICD infection was made by the clinician based on local signs of inflammation such as erythema, heat, redness or purulent drainage, outward displacement of the pocket, septicemia or infectious endocarditis of the heart. The time of infection was recorded and ICD infection was categorized

as; 'very early' if infection occurs 1 month after the latest ICD procedure; 'early' if infection occur between 2 and 12 months of the latest procedure, and 'late' if infection occurs 12 months after the latest procedure.

Ethical approval

Ethical clearance was obtained from the Institutional Review Board at the Faculty of Health Science of the University of Algeria. The administrative authorizations were obtained from the Delegation of Public Health of the Regional Hospitals of Western Algeria. Moreover, written informed consent was obtained from all study patients prior to interview and DCI collection

Collection of demographic and clinical data

Demographic and clinical data collected from each patient by direct interview included age, gender, existence of an underlying anterior cardiopathy or a history of infective endocarditis, existence of diabetes, immunosuppression, primary implantation or change of pocket, antibiotic therapy and possible infection or surgery programmed during the last five years.

Microbiological culture of the ICDs

After removal by the surgeons, the implantable cardiac devices (ICDs) were placed in sterile boxes and transported to the microbiology laboratory within 30 minutes. Each element of the ICD pocket and lead was soaked separately in sterile saline solution and sonicated for 5 minutes at a frequency of 20Khz, and vortexed for 30sec to detach and detect sessile bacteria hanging on to these devices (17,18). After sonication, 100µL of the soaking liquid was inoculated onto Chapman agar and MacConkey agar plates. The number of CFU/mL was counted after 24 hours aerobic incubation. The minimum detection threshold was 2 CFU/mL (19).

Biochemical identification and susceptibility testing of bacteria isolates

The biochemical identification of the bacteria isolates was done using the API 20E and API 20NE Staph API (BioMérieux, Marcy l'Etoile, France), and confirmed by the Siemens Healthcare Diagnostics WalkAway® 96 Plus System. The antibiotic susceptibility and resistance phenotypes were determined by the disk diffusion method on Mueller-Hinton agar (Biorad, Marnes-la-Coquette, France) according to the recommendations of the antibiogram committee of the French Society of Microbiology (20). The antibiotic disks used were penicillin (10iu), oxacillin (5µg), cefoxitin (30µg), gentamicin (10µg),

tobramycin (10µg), amikacin (30µg), vanco-mycin (30µg), rifampin (30µg), fosfomycin (50µg), fusidic acid (10µg), clindamycin (2 µg), pristinamycin (15µg), erythromycin (15 µg), ofloxacin (5µg), tetracycline (30µg), chl-oramphenicol (30µg), imipenem (10µg), and trimethoprim/sulfamethoxazole (25µg), cefo-taxime (5µg), ceftriaxone (30µg), nalidixic acid (30µg), ciprofloxacin (5µg), amoxicillin (20µg), and clavulanic acid (10µg).

Detection of biofilm by Tissue Culture Plate (TCP) method

Quantitative measurement of biofilm production in the isolates was done using a microtiter assay. In brief, cells were grown overnight in Brain Heart Infusion Broth (BHIB). The broth was then diluted 1:100 and inoculated into microtiter plates. After 24 hours incubation at 37°C, the plates were washed, stained with crystal violet, and the optical density measured at 570 nm (20). Each test was performed in triplicate. As a negative control, the uninoculated medium was used to determine the initial OD. Mean OD values were calculated for all tested strains and the negative control.

The threshold value (ODc) was estab-lished as three standard deviations (SD) above the mean OD of the negative control i. e. ODc=mean OD of the negative control + (3×SD of negative control). The ODc value was calculated separately for each microtiter plate. When a negative value was obtained, it was presented as zero, while any positive value indicated the production of biofilms. For the interpretation of the results, the classi-fication of the obtained results was based on the control OD. The strains are classified as follows: OD≤DOt (control) is non-biofilm forming; DOt×2≤OD≤DOt×4 is moderate biofilm-forming, and DOt×4≤OD is highly (strong) biofilm-forming (21).

Detection of biofilm by Congo Red Agar (CRA) method

The Congo Red Agar method was used for detecting the ability of isolate to produce slime. The CRA medium was pre-pared with 37 g/L BHI broth, 50 g/L sucrose, 10 g/L agar, and 0.8 g/L Congo Red. The Congo Red stain was prepared as a concen-trated aqueous solution and autoclaved at 121°C for 15 min separately from other medium constituents, and was then added when the agar had cooled to 55°C. The plates were inoculated with bacterial isolate and incubated at 37°C for 24 hours. A positive result was indicated by black colonies while non-producing strains developed red colonies (22). For the evaluation of colonies colors, a four-color reference scale was used according to Satorres and Alcaráz (23). Black and almost black burgundy were classified as biofilm producers, while burgundy and red as non-biofilm producing strains. This test was performed in triplicate

Hydrophobicity assays

The hydrophobicity of the bacterial wall was evaluated with the MATH protocol (24) using hexadecane as a solvent. The bacteria strains were first grown in 50 mL of Luria Bertani (LB) and incubated for 18 hours at 37°C. The cells were recuperated by centrifugation at 5000 rpm for 15 min. The pellet obtained was then washed after two successive centrifugations with PBS (Phos-phate Buffered Saline pH 7.1) and suspended in the same buffer at an initial optical density (ODi) between 0.8 and 1 at 600nm. A volume of 0.3ml of each solvent was added to 1.8ml of bacterial suspension and the mixture was vortexed for 2 min. After a 20 min settling, the optical density (ODf) of the aqueous phase was measured at 600nm and the percentage of adhesion to solvent was then calculated using the following equation; CSH %=[(ODi−ODf)/ODi]×100 (25). A CSH of 0-20% is defined as weak, 21-50% as mode-rate and > 40% as strong hydrophobicity.

Results:

From December 2012 to August 2014, a total of 315 implantable cardiac devices (ICDs) were implanted by the cardiology units of the four hospitals, out of which ICD was removed in 17 patients, all of whom developed infection on the ICDs, giving an infection rate of 5.4%. All 17 patients underwent only one surgical procedure except for one patient who had two surgical procedures within three months due to repeated infections of his ICD. The age range of the patients was 60 to 75 years; 13 (76.4%) were male and 5 (29.4%) were diabetic.

All the patients were on antibiotic therapy on the day of cardiac device insertion. Four independent predictive factors of ICD infection identified were; reinter-vention, long procedure, multi-morbidity, and ICD implantation procedure. Six patients (35.3%) had early infection of ICD while 11 patients (64.7%) had late infection. There were 9 patients with associated primary ICD implantation infections while there were 8 with change of pocket ICD infection (Table 1).

Biofilm forming bacterial infection of implantable cardiac devices

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Table 1: Distribution of bacteria strains according to the implantable cardiac device (ICD)

Pacemaker	Isolated strains		Hospital	Gender (M/F)	Duration of the intervention	Primo or change of pocket (P/C)	Endocarditis
	Pocket	Lead					
P1	Negative	S1: *Moraxella*	CHU Tlemcen	F	50min	P	
		S'1: *Staphylococcus xylosus*					
P2	P'2: *Staphylococcus xylosus*	Negative	CHU Tlemcen	M	1h 20 min	C	
P3 (DAI)	P3: *Pseudomonas aeruginosa*	S3: *Enterobacter cloacae*	EH Ain Temouchent	M	7h	P	
		S3p: *Pseudomonas aeruginosa*					
P4	P'4: *Staphylococcus épidermidis*	S'4: *Staphylococcus épidermidis*	EH Ain Temouchent	M	4hs	P	
P5	P'5: *Staphylococcus sciuri*	S5: *Proteus penneri*	CHU Tlemcen	M	1H30 min	P	
P6	P6: *Pseudomonas aeruginosa*	S6: *Vibrio fluviatis*	CHU Tlemcen	F	1h 10 min	C	Positive
	P'6: *Staphylococcus sciuri*	S'6: *Staphylococcus capitis*					
P7	P'7: *Staphylococcus sciuri*	S'7: *Staphylococcus sciuri*	CHU Tlemcen	M	25 min	C	
	P8: *Vibrio fluviatis*	S8: *Vibrio vulgaris*	CHU Tlemcen	M	1h 15 min	P	
P8	P'8: *Staphylococcus sciuri*	S'8: *Staphylococcus épidermidis*					
P9	P9: *Pseudomonas horyzihabitans*	S9: *Pseudomonas aeruginosa*	EH Ain Temouchent	M	20 min	P	
	P'9: *Staphylococcus simulans*	S'9: *Staphylococcus haemolyticus*					
	P10: *Proteus penneri*	S10: *Proteus penneri*	CHU Tlemcen	F	1h 10 min	C	
P10	P''10: *Chrysobacterium indolegenes*	S'10: *Staphylococcus warneri*					
	P'10: *Staphylococcus hominis*						
P11	P11 : *Pasteurella pneumotropica*	S11: *Ochrabactrum antropi*	CHU Tlemcen	M	1h 15min	C	
	P'11: *Staphylococcus lugdunensis*	S'11: *Staphylococcus épidermidis*					
P12	P12: *Ewingella americana*	S12: *Ewingella americana*	CHU Tlemcen	M	1h	P	
	P'12: *Staphylococcus epidermidis*	S'12: *Staphylococcus epidermidis*					
P13	P13: *Pseudomonas aeruginosa*	S13: *Vibrio fluviatis*	CHU Tlemcen	M	20 min	C	Positive
	P'13: *Staphylococcus hominis*						
P14	P14: *Photobacterium damselae*	S14: *Photobacterium damselae*	CHU Tlemcen	M	25min	C	
P15	P15: *Serratia liquefaciens*	S15: *Enterobacter aerogenes*	Kara Clinic	M	1h	C	Positive
		S'15: *Staphylococcus épidermidis*					
P16	P16: *Serratia plymuthyca*	S16: *Photobacterium damselae*	CHU Tlemcen	F	55min	P	Positive
	P''16: *Pasteurella pneumotropica*	S'16: *Staphylococcus hominis*					
	P'16: *Staphylococcus hominis*						
P17	P17: *Photobaterium damselae*	S17: *Moraxella*	CHU Tlemcen	H	1h 15min	P	
	P'17: *Staphylococcus épidermidis*	S'17: *Staphylococcus épidermidis*					

CHU: University Hospital Centre   EH: Hospital / Health Institution   Kara clinic: Private Clinic

All 17 ICDs (pockets and leads) were positive for bacteria on culture plates, and 54 bacteria (26 Gram positives and 26 Gram-negatives) were isolated and identified, with the most frequent being coagulase-negative staphylococci (CoNS) representing 46.3% (25 /54) of the total isolates (Table 2 and 3). A total of 27 bacterial isolates were recovered from the ICD pockets and 27 from the ICD leads. The most frequently isolated enteric bacteria were *Pseudomonas* spp, *Klebsiella* spp, *Proteus* spp., *Serratia* spp, and *Entero-*

bacter spp identified in 9 ICDs comprising 29 out of the 54 (53.7%) strains.

Other infrequently isolated bacteria from some ICDs included *Ochrabactrum antropi*, *Ewingella americana*, and *Photo-bacterium damselae* which were not asso-ciated with infectious endocarditis in the patients. Polymicrobial infection was recorded in 15 of the 17 patients (88.2%) with varying combinations of *Pseudomonas aeruginosa*, *Staphylococcus sciuri, Vibrio fluviatis* and *Staphylococcus capitis* while the infection was monomicrobial in a patient (P4) where the ICD pocket and the lead were colonized by the same *Staphylococcus epidermidis* strain and another patient (P2) where only the pocket was colonized by *Staphylococcus xylosus* (Table 1).

Table 2: Distribution of Gram-negative bacteria strains according to the implantable cardiac device (ICD)

Strains	Production of slime	Tissue culture plate	MATH	Antibiotype
S1 *Moraxella*	Non- Producer	Strong		na^R amp^R, amc^R cro^R ctx^R kf^R
P3 *Pseudomonas aeruginosa*	Non- Producer	Strong	Strong	caz^R ctx^R, tcc^R cn^I
S3 *Enterobacter cloacae*	Producer	Strong	Strong	cn^R c^R ak^I ff^I cro^R te^R amc^R tob^R fa^R ctx^R amp^R
S3p *Pseudomonas aeruginosa*	Producer	Strong	Strong	tob^I na^R cn^R te^R amc^R cro^R kf^R amp^R ctx^R caz^R
S5 *Proteus penneri*	Producer	Strong		amc^R amp^R kf^R ipm^R ctx^R cro^R ofx^R cip^R na^R cn^R tob^R te^R
P6 *Pseudomonas aeruginosa*	Producer	Strong	Weak	tob^R ak^I na^R cn^R te^R amc^R cro^R kf^R amp^R ctx^R caz^R
S6 *Vibrio fluviatis*	Producer	Strong		na^R te^I amc^R cro^I kf^R amp^R ctx^R
P8 *Vibrio fluviatis*	Producer	Strong		na^R amc^R kf^R amp^R ctx^R
S8 *Vibrio vulgaris*	Producer	Strong		ak^R na^R cn^R te^R amc^R cro^R kf^R amp^R ctx^R caz^R
P9 *Pseudomonas horyzihabitans*	Producer	Strong	Weak	tob^R ak^R na^R cn^R te^R amc^R cro^R kf^R amp^R ctx^R
S9 *Pseudomonas aeruginosa*	Producer	Strong	Moderate	cip^I ofx^R tob^R ak^R na^R cn^R te^R amc^R cro^R kf^R amp^R ctx^R
P10 *Proteus penneri*	Producer	Strong		cip^R ofx^R na^R ctx^I
P''10 *Chrysobacterium indolegenes*	Producer	Strong		cip^R ofx^R tob^I na^{R}cn^I te^{R}amc^I kf^R ctx^R
S10 *Proteus penneri*	Producer	Strong		na^I amc^R cro^R kf^R amp^R ctx^R
P11 *Pasteurella pneumotropica*	Producer	Strong		na^R amc^R kf^R amp^R ctx^R cro^R
S11 *Ochrabactrum antropi*	Producer	Strong	Weak	na^R amc^R kf^R amp^{R}ctx^R ipm^R
P12 *Ewingella americana*	Producer	Strong	Strong	ctx^R cro^R amp^R fa^I amc^R tob^R
S12 *Ewingella Americana*	Producer	Strong	Strong	ctx^R cro^R amp^R fa^I amc^R tob^R
P13 *Pseudomonas aeruginosa*	Producer	Strong	Strong	ctx^R cro^R amp^R c^R fa^R caz^R ff^I te^R amc^R tob^I
S13 *Vibrio fluviatis*	Non- Producer	Strong		ctx^R cro^R amp^R fa^I cip^I ff^R ofx^I te^R amc^R tob^R ipm^R
P14 *Photobacterium damselae*	Producer	Strong	Weak	ctx^R cro^R amp^R te^R amc^R
S14 *Photobacterium damselae*	Producer	Strong	Weak	ctx^R cro^R amp^R te^R amc^R
P15 *Serratia liquefaciens*	Producer	Strong	Strong	ctx^R cro^R amp^R fa^R ff^I te^R amc^R tob^R
S15 *Enterobacter aerogenes*	Producer	Strong	Strong	ctx^R cro^R amp^R cn^R kf^R te^R amc^R tob^R caz^R
P16 *Serratia plymuthyca*	Non- Producer	Strong	Strong	amc^I cro^R kf^R amp^R ctx^R
S16 *Photobacterium damselae*	Producer	Strong	Weak	cn^I tob^I amc^R cro^R amp^R ctx^R
P17 *Photobaterium damselae*	Producer	Strong	Weak	tob^I cro^I
S17 *Moraxella*	Non- Producer	Strong	Strong	ctx^R cro^R amp^R te^I amc^R
P''16 *Pasteurella pneumotropica*	Non- Producer	Weak		cn^I tob^I amc^R cro^R kf^R amp^R ctx^R

na=nalidixic acid; amp=ampicillin; amc=amoxicillin; cro=ceftriaxone; ctx=cefotaxime; caz=ceftazidime; te=tetracycline; tob=tobramycin; fa=fusidic acid; ff=fosfomycin; ipm=imipenem; cip=ciprofloxacin; ofx=ofloxacin; cn=chloramphenicol

Table 3: Distribution of Gram-positive bacteria strains according to the implantable cardiac device (ICD)

Strains	Production of slime	Tissue culture plate	Math	Antibiotype
P'2 *Staphylococcus xylosus*	Non- Producer	Strong		$na^I k^R va^I$
P'9 *Staphylococcus simulans*	Producer	Strong	Strong	$na^R va^I te^R$
P''10 *Chrysobacterium indolegenes*	Producer	Strong		$cip^R ofx^R tob^I na^R cn^I te^R amc^I kf^R ctx^R$
S'1 *Staphylococcus xylosus*	Producer	Moderate	Weak	$na^I k^R va^I$
P'4 *Staphylococcus épidermidis*	Non- Producer	Moderate	Strong	$ox^R tob^R cn^R va^I ox^R$
S'4 *Staphylococcus épidermidis*	Non- Producer	Moderate		$ox^R ra^S tob^R cn^R va^I ox^R$
P'5 *Staphylococcus sciuri*	Producer	Moderate	Strong; Hydrophobe	$na^R amc^R cro^I amp^R ctx^R kf^R va^I$
S'6 *Staphylococcus capitis*	Producer	Moderate	Moderate	$na^R amc^R cro^I amp^R ctx^R kf^R va^I$
P'7 *Staphylococcus sciuri*	Producer	Moderate	Weak	$na^I c^I va^I te^R$
S'7 *Staphylococcus sciuri*	Producer	Moderate	Weak	$na^I c^I va^I te^R$
S'8 *Staphylococcus épidermidis*	Producer	Moderate	Weak	$ox^I na^R c^R va^I te^R tob^I$
P'10 *Staphylococcus hominis*	Producer	Moderate	Strong; Hydrophobe	$cro^I kf^I ctx^R$
S'10 *Staphylococcus warneri*	Producer	Moderate	Weak	$na^I amc^R cro^I kf^R amp^R ctx^R ff^I$
P'11 *Staphylococcus lugdunensis*	Producer	Moderate	Weak	$na^I kf^I amp^I ctx^R$
S'11 *Staphylococcus épidermidis*	Producer	Moderate	Strong	$na^I amc^R cro^R kf^R amp^R ctx^R$
P'12 *Staphylococcus epidermidis*	Producer	Moderate	Weak	$ox^R na^R n^I va^I te^R tob^R$
P'13 *Staphylococcus hominis*	Non- Producer	Moderate	Weak	$va^I te^R$
S'15 *Staphylococcus épidermidis*	Producer	Moderate	Weak	$ox^R na^S pnv^R n^S c^S k^S cip^S pef^S va^I te^R ra^R tob^R$
P'16 *Staphylococcus hominis*	Producer	Moderate	Weak	$na^I va^I$
S'16 *Staphylococcus hominis*	Producer	Moderate		$ox^I na^R n^I va^I te^R$
P'17 *Staphylococcus warneri*	Producer	Moderate	Strong	$na^I va^I$
P'6 *Staphylococcus sciuri*	Producer	Weak	Weak	$na^R va^I$
P'8 *Staphylococcus sciuri*	Producer	Weak	Weak	$tob^I ak^I na^R cn^R te^R amc^R cro^R kf^R amp^R ctx^R$
S'9 *Staphylococcus haemolyticus*	Producer	Weak	Strong; Hydrophobe	$na^R amc^R cro^R kf^R amp^R ctx^R$
S'12 *Staphylococcus chromogenes*	Producer	Weak	Weak	$na^I k^R va^I te^I tob^R$
S'17 *Staphylococcus épidermidis*	Producer	Weak	Weak	$na^I k^R va^I tob^R$

na=nalidixic acid; tob=tobramycin; va=vancomycin; cn=chloramphenicol; te=tetracycline; cro=ceftriaxone; ctx=cefotaxime; amp=ampicillin; amc=amoxicillin; ox=oxacillin; ff=fosfomycin; cip=ciprofloxacin; pnv=pristinamycin; pef=pefloxacin

This study of antibiotic susceptibility of the isolated strains in the four hospital centers showed resistance at a significant level against several antibiotics (Fig 1 and Fig 2). Fig 1 is a histogram illustrating the resistant pattern of the Gram-negative bacterial isolates showing high resistance rate (>90%) to amoxicillin, clavulanic acid, cefotaxime, and gentamycin, with 3 isolates resistant to imipenem. None of the staphylococcal strains was resistant to vancomycin. Fig 2 is a histogram illustrating the resistance pattern of the Gram-positive isolates.
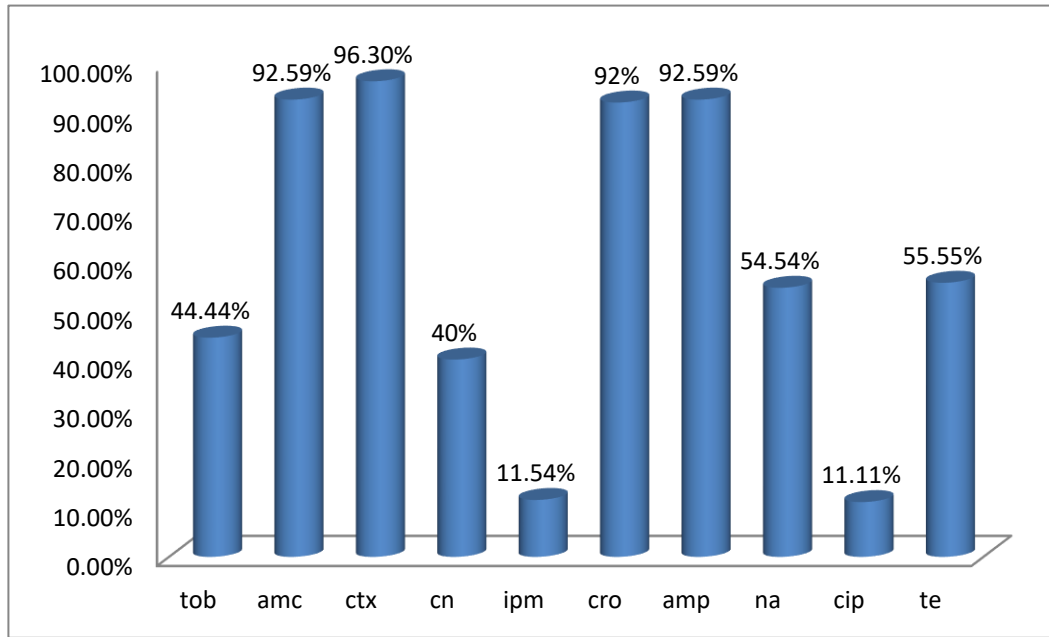

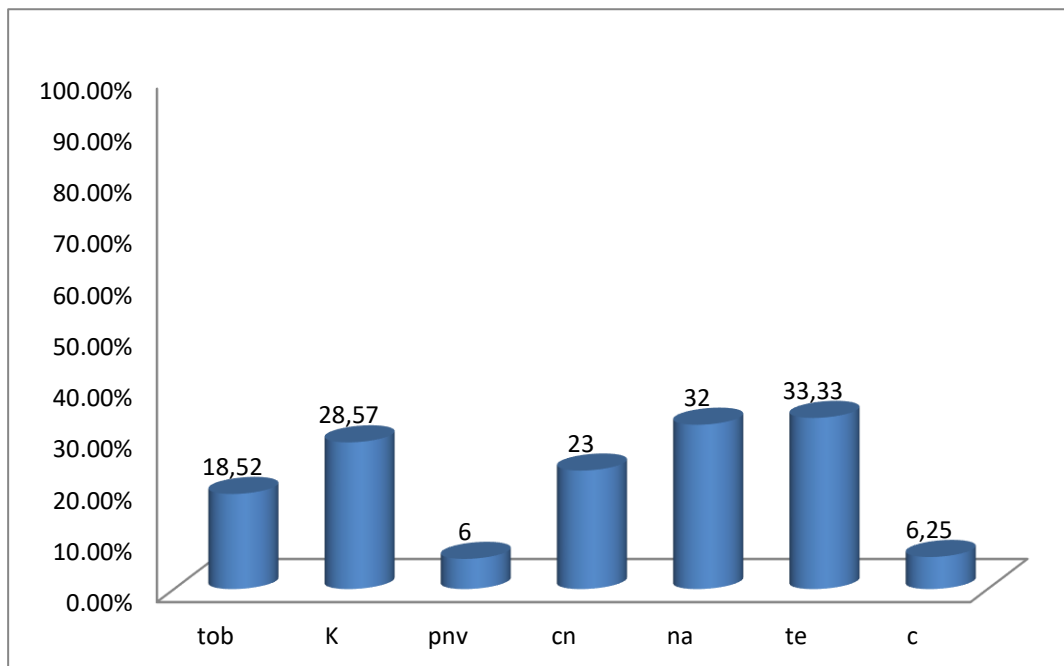Fig 1: (Histogram A$_1$): Resistance rate of Gram-negative bacteria isolates

Fig 2: (Histogram A$_2$): Resistance rate of Gram-positive bacteria isolates

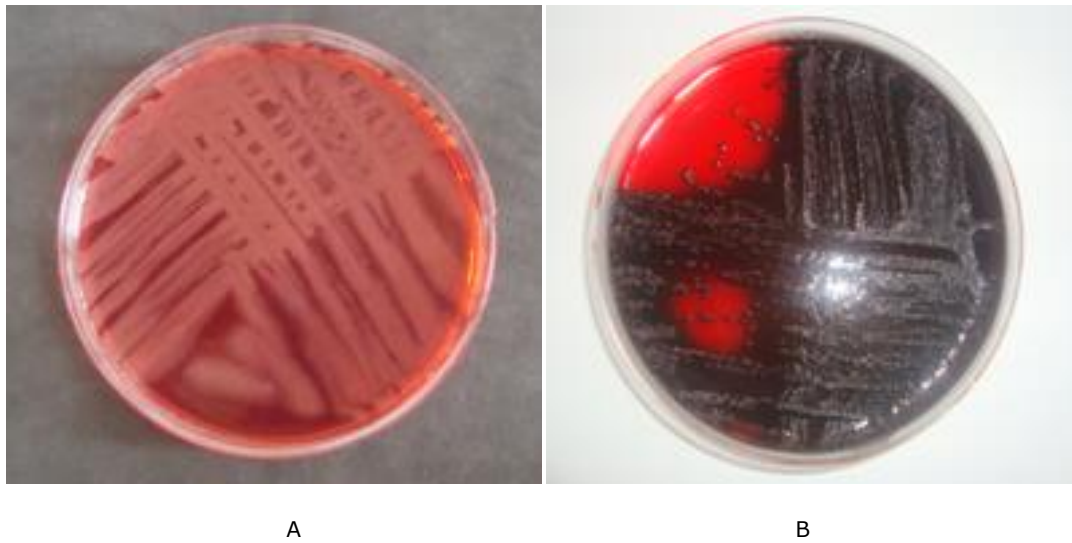A   B

Fig 3: Slime production in the strains isolated on Congo red medium
A: slime generating, B: non-slime generating

Results of biofilm detection

The results of the biofilm production by the bacterial isolates with the qualitative CRA and the quantitative TCP methods are presented in Table 2. Out of the 54 bacteria isolates, 44 (81.5%) were slime producers by the CRA method and 24 of them were strong (very good) biofilm producers using the TCP method, with OD 570 > 0.240. The TCP method detected biofilms in 48 (88.9%) of 54 isolates with different intensities; 30 (55%) isolates were strong producers, 18 (33.3%) isolates were moderate, 6 (11.1%) isolates were low biofilm producers, while 10 (18.5%) were non-producers of biofilms. Thirty-seven (68.5%) of the 54 isolates were positive by both TCP and RCA methods. Fig 3 is a photograph of CRA plate showing slime prod-

uction by a positive isolate (A) and non-slime production by a negative isolate (B).

Result of hydrophobicity

The results of the microbial adhesion to the solvent (hydrophobicity) are summarized in Figs 4 and 5. A total of 14 Gram positive and 7 Gram negative bacteria isolates had affinity to hexadecane (apolar solvent) suggesting a weak hydrophilic character. On the other hand, 17 (41.5%) isolates were relatively hydrophobic between 1% and 96%, and 2 (5%) strains were moderately hydrophobic. Fig 4 is a histogram of the cell surface hydrophobicity of the Gram-negative bacteria isolates and Fig 5 is a histogram of the cell surface hydrophobicity of Gram-positive bacteria isolates.
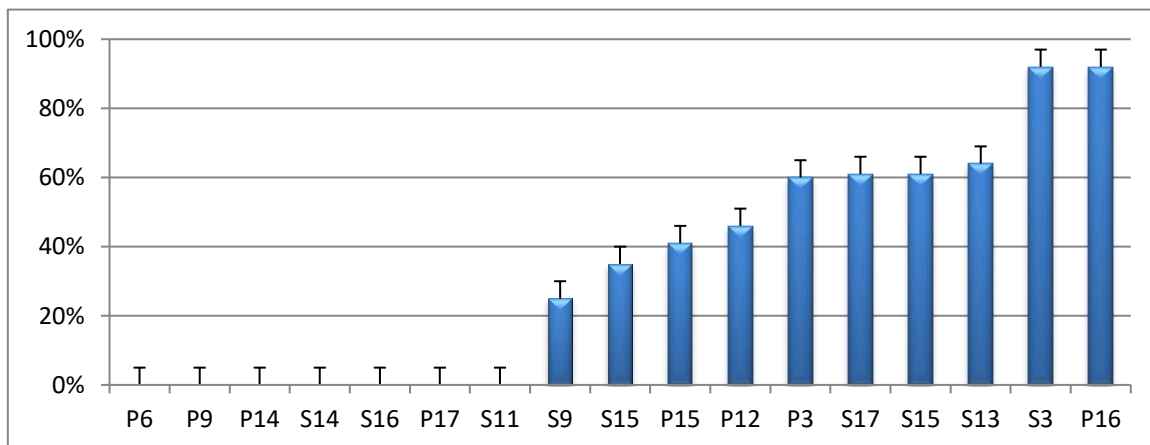

Fig 4: (Histogram B_1): Cell surface hydrophobicity of Gram-negative bacteria isolates
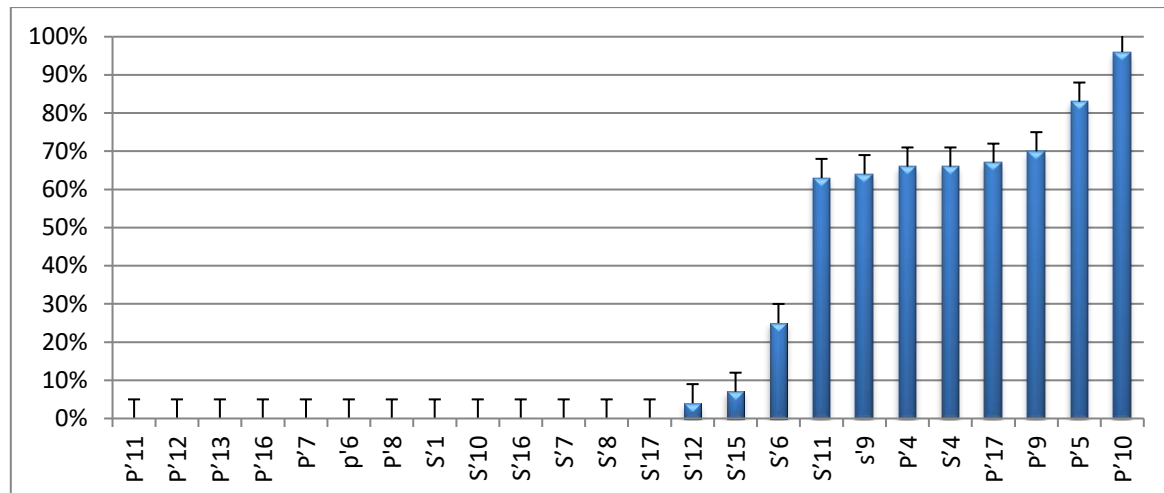
Fig 5: (Histogram B$_2$): Cell surface hydrophobicity of Gram-positive bacteria isolates

Discussion:

ICDs have become increasingly of great importance in the management of heart disease in many countries around the world with a great impact on the quality of life of patients (10). The advances in the development of permanent pacemakers and the technologies of implantable cardiac defibrillators have helped this process. Nevertheless, with the increase in ICD implantations, infections on these devices have correspondingly increased. To the best of our knowledge, this is the first and only study on the microbiological characteristics of ICD infections in Algeria. Although our data reflect the epidemiology of ICD infections in the region, they could be of great interest and provide useful information for ICD infections management in other regions.

In recent decades, the rate of these ICD infections has increased worldwide from 0.13% to 19.9% with an average rate of 10% (15,26). The overall incidence of infection on ICD implantable cardiac devices is estimated at 1.9 per thousand per year (27). According to an estimate by Camus et al., there are more than three million patients on pacemaker (PM) worldwide and 180,000 carriers of implantable defibrillator (ID) (28). Among the complications occurring after the implantation of an ICD, infection is certainly the most serious (29). In this retrospective study of 21 months, of a total of 315 ICD implantations in 4 hospitals, 17 patients presented with infection of their ICDs, giving an incidence rate of 5.4%. All the infections were diagnosed and confirmed by specialist cardiologists with clinical presentations that included erythema, heat, redness with or without purulent drainage, sepsis and infectious endocarditis. This rate is quite high when compared to other studies such as the French study by Klug and his team (30) in 2000 where the incidence of infectious complications was only 0.68%. In 2010, Baddour et al., (31) and Voigt et al., (32) estimated the incidence to be between 0.13% and 20%, which was related to the increase in the implantation rate of ICDs in the world in general. Of the 17 infected patients, 16 had infection of their pacemakers (PM), giving an incidence rate of 5.1% in the study. In the literature, the incidence of pacemaker infections is known to vary between 0.13% and 19.9% occurring mostly at the site of implantation of the generator, and in 10% of cases, it is the cause of infectious endocarditis (33). Only one patient had an infection after defibrillator implantation (DI) representing an incidence of 0.3%, which is known to vary between 0.7% and 1.2% (34).

The infection of an ICD pocket is a rare but much feared early complication, which is defined as an infection occurring within the six months following the procedures (35) and often accompanied by fever and local signs that require urgent antibiotic therapy and immediate removal of the device (36). In our study, 6 of the 17 (35.3%) infected patients had early infection of ICD. According to the study by Ben Abid et al., (37), 25% of ICD infections are early and 42% are late, with no significant difference reported with respect to the type of infectious organisms. Nine of the 17 (52.9%) infected patients had an outward displacement of the pocket, a rate that is considerably higher

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compared to the study of Klug et al., (38) where 29.5% of the patients presented with an outward displacement of the pocket in a study of 105 patients.

There are various factors predisposing to ICD infection that have been reported including advanced age, diabetes, cancer and immunosuppression, anticoagulation, presence of a temporary stimulation lead, duration of intervention, and the surgeon's experience (39). Indeed, diabetes mellitus and age are predisposing factors in most of our patients. The highest rate of infection was observed in the age group 60 to 75 years who were mainly male patients among whom 29.4% were diabetic. A total of 15 patients had positive cultures on the lead and the pocket of their ICDs, 8 of whom were infected by the same bacterium. Victor et al., (40) reported that the infection of the lead is associated with that of the pocket in every two cases. This infection can occur in isolation and in one fourth of cases a few weeks after implantation (40). The culture of the removed devices allows the possibility of an etiological diagnosis of ICD infection in the vast majority of cases. This means that microbiological analysis is mandatory in the case of suspicion of an ICD infection.

A total of 54 bacteria were isolated and identified from the ICDs; 46.3% Gram positive bacteria were isolated, which were mostly CoNS with predominance of *S. epidermidis*. These findings closely agree with to those reported by Gill et al., (41) who estimated that about 41.2% of ICD infections were caused by CoNS. The dominant role of staphylococci is consistent with what has been previously reported where staphylococcal species accounted for 60 to 80% of the cases in most of the reported studies (36,42,44-44). In several studies, *S. epidermidis* was the most frequently isolated CoNS species and constitutes a significant part of the normal bacterial flora of human skin and mucous membranes from where it is easily introduced as a contaminant during surgical implantation of the polymer device (45).

Gram-negative bacteria constituted 53.7% of the isolated bacteria, with high frequency of enteric bacteria such as *Proteus* spp, *Serratia* spp, *Enterobacter* spp and *Pseudomonas* spp. These bacteria which are involved contamination and care-related infections were recovered twice in the space of a few weeks in two different hospitals. This implies either contamination or poor technique during the surgical procedure. We noted the exceptional recovery of three uncommon bacteria species; *Ewingella ameri-*

cana, *Ochrabactrum antropi,* and *Photobacterium damselae* from the ICDs in our study, which have been reported only by a few authors such as Pien et al., (46), Mahmood et al., (47), and Austin et al., (48). Nevertheless, the important causative microorganisms of ICD infections are CoNS followed by Gram-negative bacilli, fungi, non-tuberculous mycobacteria and *Bulkholderia cepacia.* The bacterial complex is a rare cause of ICD infection with only a few cases of ICD pocket infection reported in the literature (41,49,50-51). ICD infections are mainly caused by the contamination of the local bacterial flora during implantation. Skin micro-organisms migrate from the insertion site along surface of the lead, colonize the intravascular distal portion, and finally infect the blood (52).

Nearly all of the isolated Gram-negative bacteria in this study were resistant to ampicillin, amoxicillin, cefotaxime, and ceftriaxone. Although imipenem was highly active on the tested strains, three isolates were resistant to this antibiotic. These strains were also resistant to quinolones with rates ranging from 11.1% for ciprofloxacin to 54.5% for nalidixic acid. Resistance rates of isolates to tetracycline and tobramycin were 55.5% and 44.4% respectively. Unlike Gram-negative bacteria, CoNS isolates displayed a higher level of sensitivity but with some were resistant to nalidixic acid and tetracycline. According to 2008 Camus study (28), majority of staphylococci are multi-sensitive with especial sensitivity to aminoglycosides, but the isolates in our study were resistant to kanamycin (28.5%) and tobramycin (18.5%).

The development and persistence of bacterial infections are often associated with the foreign equipment and materials used in the devices and the ability of these bacteria to adhere to them. The bacterial strains implanted on the surface of a device are inserted and protected by a dense polysaccharide extracellular matrix and are more resistant to antibiotics, constituting the biofilm that can be detected by quantitative and qualitative techniques (1). Adherence and persistence of CoNS infection are often associated with foreign materials. Adhesion is primarily related to non-specific physico-chemical forces, and then to specific interaction of the bacterial surface adhesins (not directly with the device) with host proteins coating the device (53). CoNS may adhere directly to the device plastic polymers via fimbria-like surface protein structures or via a capsular polysaccharidic adhesin (54). Bacteria may also adhere to host matrix proteins such as fibrinogen, fibronectin, and

collagen that coat the surface of an implanted device (55). The layers of bacteria on the surface of an implanted device are encased in an extracellular slime made of a polysaccharide intercellular adhesion and constitute the biofilm (56,57). Microbes in a biofilm are protected by this dense extracellular matrix and are more resistant to antibiotics and host defenses. Biofilm forming bacteria are therefore a public health challenge for those requiring indwelling medical devices (56).

In our study, the presence of infection was influenced by the ability of 48 out of 54 strains to form a biofilm, whatever the support (pocket and leads), and thus representing a significant risk factor. The TCP technique, which is the most widely used method, is considered the 'gold standard' test for sensitive, accurate and reproducible screening method for the detection of biofilm production in clinical isolates (58). The adherence capacity of clinical isolates to host cells depends on bacterial surface properties such as hydrophobicity. In a recent study, it was reported that there is a significant difference in cell surface load between group B streptococci treated with antibiotics and those untreated (59). The determination of the acid-base properties (acceptor electron, donor electron) may be of great importance in many research areas of phagocytosis and microbial adhesion (60).

Our data indicated that affinity to hexadecane (apolar solvent) suggests low hydrophilicity for the majority of the bacteria isolated from surfaces of hydrophobic medical device. This contradicts several studies which reported that hydrophobic cells tend to adhere to hydrophobic substrates, while hydrophilic cells tend to adhere to hydrophilic substrates (61,62). It has also been suggested that there is a positive correlation between the degree of bacterial hydrophobicity and adhesion to abiotic surfaces (63). Our finding can be explained by the fact that these bacteria adhere to host proteins such as fibrinogen, fibronectin, and collagen which cover the surface of the implanted device, making it hydrophobic (55). The comparison between the biofilm formation (by RCA and TCP), hydrophobicity and antimicrobial resistance of the 54 bacteria isolates revealed that there is no significant association in terms of isolates but that there is correlation between biofilm formation and resistance to antibiotics, with the strong biofilm producers resistant to more than one antibiotic.

Conclusion:

This study is the first in Algeria to describe microbiological characteristics of ICD infections. CoNS were the most frequent cause of ICD infections in our study but *Pseudomonas aeruginosa* and *Serratia* spp were also significant cause of bacteremia in patients with ICD, with comparable rate to the CoNS. This situation may imply bacteria contamination or poor technique during surgical procedure for implantation of the ICDs.

The bacteria in the biofilm were protected, more resistant and tolerated high concentrations of antibiotics, and thus played a major role in the development of ICD infections. Despite the improvements in ICD design and implantation techniques, ICD infection remains a serious challenge. It is therefore essential that proper infection prevention and control practices be put in place as well as strict indications for antibiotic prophylaxis during implantation of ICDs.

Conflict of interest statement:

No conflict of interest is declared.

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