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Comparative study of the action of fluoroquinolones and vancomycin on biofilms of *Staphylococcus* strains isolated from infections of peritoneal dialysis catheters

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Abstract:

Background: catheter mediated infections are mainly caused by Staphylococci, the treatment of these infections is challenging because of biofilm formation.

Methods: A microtiter plate biofilm assay was used to study the in vitro activity of Vancomycin, Ofloxacin and Levofloxacin on preformed biofilms of 30 stains of *Staphylococcus* isolated from peritoneal dialysis catheters and fluids at concentrations like those used intraperitoneally.

Results: The in vitro action of the three antibiotics on preformed biofilms was statistically significant. it was significantly higher for Ofloxacin and Levofloxacin rather than that of Vancomycin.

Discussion: the results demonstrate the effectiveness of the three antibiotics at concentrations like those used intraperitoneally and the superiority of Fluoroquinolones activity on Staphylococcal biofilms, these results correlate with the conclusions of other authors.

Keywords: *Biofilm; fluoroquinolones; vancomycin; peritoneal dialysis; catheters.*

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Introduction

Infections occurring in dialysis patients are much more frequent than in general population (100 times) [10]. These infections can compromise the dialysis technique or even be life-threatening; they are the second leading cause of death among dialysis patients [1]. Staphylococci are the most common cause of catheter mediated infections (45-60% of cases) [1]. The treatment of these infections is problematic, in addition to the increased antibiotic resistance of isolated bacterial strains, antibiotic therapy also runs up against the formation of biofilm, especially by staphylococcus strains; various factors have been suggested to be responsible for the resistance of biofilm to antibiotic therapy: slow growth, the presence of an exopolysaccharide matrix that can reduce the diffusion of antibiotics, and the presence of persistent bacteria. [12]

Although vancomycin remains a major anti-staphylococcal drug, its action on biofilms remains controversial [15, 11, 2], [7,6]

The aim of this study was to investigate the antibiotic therapy for staphylococcal biofilm-associated infections by comparing the in vitro action of Fluoroquinolones (Ofloxacin and Levofloxacin) and Vancomycin on a few *Staphylococcus* isolates obtained from samples of peritoneal dialysis fluids or catheters.

Methods

We studied 30 isolates of *Staphylococcus species* (15 *Staphylococcus aureus* and 15 coagulase negative *Staphylococci*). These isolates were obtained from samples of peritoneal dialysis fluids or catheters of 24 patients at the HUSSEIN DEY Hospital in Algiers. We evaluated the action of three antibiotics: Vancomycin, Ofloxacin and Levofloxacin on the preformed biofilms.

The choice of antibiotic concentrations was based on the concentrations used by intraperitoneal route according to the recommendations of the International Society for peritoneal dialysis (ISPD) [9], for each antibiotic we opted for the median concentration between the loading dose concentration and the minimum concentration at which the maintenance dose is administered, that minimum concentration is equal to "the concentration of loading dose"-"the maintenance dose concentration" so the concentrations used were calculated by the following formula:

$$C = \frac{2 * C_{\text{loading dose}} - C_{\text{maintenance dose}}}{2}$$

The loading dose concentration for Vancomycin is 1000 mg/l while the maintenance dose concentration is 25mg/l, so the concentration of Vancomycin used in this protocol using the formula above is 987.5mg/l.

The loading dose concentration for Fluoroquinolones is 50 mg/l while the maintenance dose concentration is 25 mg/l, so the concentration of Fluoroquinolones used in this protocol is 37.5 mg/l.

Study protocol

To evaluate the activity of these antibiotics on biofilm, we conducted this evaluation based on microtiter plate biofilm assay protocols proposed by George A. O'Toole [3] and J.H.Merritt et al. (microtiter plate biofilm assay) [4], we opted for the following protocol:

1. Dilution to 1/100 (10µl /990µl) of suspensions (at 0.5McF) of each bacterial strain in a liquid BHIB (Brain Heart Infusion Broth) medium.
2. Distribution of 100 µl of diluted bacterial suspensions on microplates at a rate of 4 wells per strain for a total of 120 wells for the 30 strains.
3. Incubation of microplates (covered with a film to avoid desiccation) for 24 hours at 37°C to induce the formation of biofilms on the polymer of microplates.
4. Three washes were carried out with sterile physiological water to eliminate the planktonic forms of bacteria.
5. For each bacterial strain: a well was incubated with a liquid medium (BHIB) with no additives (control well), a well with a vancomycin solution at 987.5mg/l in BHIB, a well with an Ofloxacin solution and a well with a Levofloxacin solution at 37.5 mg/l in BHIB, at a rate of 100 µl for each solution.
6. Microplates were incubated with these suspensions for 24 Hours at 37°C, avoiding desiccation.
7. Three washes were carried out with sterile physiological water and then the wells were dried at room temperature.
8. The biofilm was quantified using a gentian violet solution at 1%:150 µl per well with an incubation for 15 minutes.
9. Three washes were carried out to remove the excess of the dye and then the wells were dried for 3 hours at room temperature.
10. 200µl of 95° ethanol was added for each well.
11. Incubate ethanol in wells for 10 minutes, avoiding desiccation. This will allow the solubilization of gentian violet.
12. The contents of the microplates were transferred to new flat-bottom microplates.
13. Optical Densities (OD) were measured using a plate reader at a wavelength of 595 nm.
14. The measured optical density is correlated with the persistent biofilm mass in each well.

- To compare the OD obtained for each one of the 30 strains after the different treatments on the preformed biofilms, we used the statistical Student Test of paired values (on STHDA software), comparing the data in pairs (control/Levofloxacin, vancomycin/Ofloxacin,...).

Results

The optical density obtained from each well group according to the applied treatment (Controls, Vancomycin, Ofloxacin, Levofloxacin) for the 30 isolates of *Staphylococcus spp* are shown in Figure 1 and Table 1.

Table (1): optical densities proportional to biofilm densities of the 30 isolates of *Staphylococcus sp* before and after different antibiotic treatments.

Strains	1	2	3	4	5	6	7	
controls	0,378	0,385	0,374	0,121	0,138	0,321	0,081	
VANCOMYCINE	0,317	0,149	0,275	0,083	0,136	0,099	0,080	
LEVOFLOXACINE	0,293	0,079	0,115	0,099	0,070	0,057	0,080	
OFLOXACINE	0,228	0,115	0,115	0,092	0,064	0,062	0,079	
Strains	8	9	10	11	12	13	14	15
controls	0,283	0,149	0,355	0,243	0,085	0,241	0,185	0,128
VANCOMYCINE	0,094	0,100	0,218	0,242	0,079	0,134	0,098	0,016
LEVOFLOXACINE	0,137	0,148	0,154	0,241	0,076	0,085	0,094	0,110
OFLOXACINE	0,104	0,067	0,203	0,183	0,070	0,096	0,128	0,105
Strains	16	17	18	19	20	21	22	23
controls	0,101	0,257	0,141	0,151	0,168	0,247	0,188	0,141
VANCOMYCINE	0,100	0,209	0,140	0,144	0,131	0,210	0,180	0,140
LEVOFLOXACINE	0,098	0,107	0,134	0,109	0,100	0,109	0,160	0,016
OFLOXACINE	0,089	0,134	0,114	0,087	0,116	0,145	0,171	0,125
Strains	24	25	26	27	28	29	30	moyennes
controls	0,145	0,133	0,085	0,116	0,130	0,130	0,101	0,190
VANCOMYCINE	0,082	0,113	0,084	0,097	0,089	0,101	0,100	0,135
LEVOFLOXACINE	0,095	0,130	0,080	0,110	0,128	0,112	0,084	0,114
OFLOXACINE	0,076	0,132	0,084	0,115	0,127	0,096	0,100	0,114

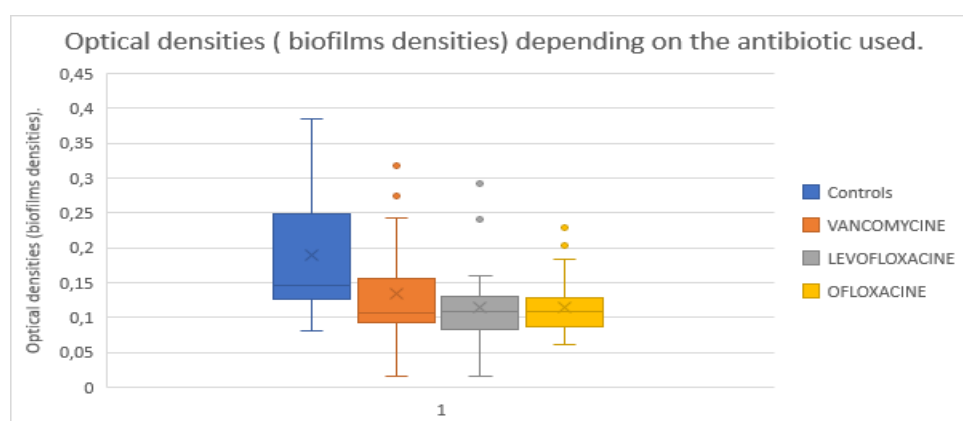


Fig (1): Distribution of OD values in relation to biofilms according to the antibiotic treatment applied. (crosses (X) represent the average OD for each type of well: controls, Vancomycin, Levofloxacin, Ofloxacin. Horizontal lines represent median OD).

The average of optical density for the control well group was 0.190, while for treated wells the average was 0.135 for vancomycin, and 0.114 for Ofloxacin and Levofloxacin.

The action of the 3 antibiotics on the preformed biofilms of the 30 isolates studied was statistically significant (STUDENT test of paired values) compared to controls wells: for vancomycin $p=0.00088$ ($t=4.5489$, $df=29$), for ofloxacin $p=0.000019$ ($t=5.10$, $df=29$) and for Levofloxacin $p=0.000059$ ($t=4.695$, $df=29$).

The action of Ofloxacin and Levofloxacin on staphylococcal biofilms were significantly higher than that of Vancomycin $p=0.019$ ($t=2.47$, $df=29$) and $p=0.042$ ($t=2.12$, $df=29$) respectively. On the other hand, the activity of ofloxacin and Levofloxacin

on the biofilms of our isolates was similar $p=0.95$ ($t=0.06$, $df=29$).

Discussion

A microtiter plate biofilm assay based on the use of a dye and the measurement of optical density to quantify biofilms was used to evaluate the activity of Fluoroquinolones (Ofloxacin and Levofloxacin) and Vancomycin on 30 *Staphylococcus sp* isolates at concentrations like those used intraperitoneally. The measured optical density at 595 nm is correlated with the persistent biofilm mass in each well, therefore for each of the isolates: the lower the optical density compared to the control well, the greater the antibiofilm activity of the antibiotic.

The action of the three antibiotics on the preformed biofilms was statistically significant (STUDENT test of paired values) compared to controls wells. The action of Ofloxacin and Levofloxacin on biofilms were significantly higher than that of Vancomycin, and finally the activity of Ofloxacin and Levofloxacin was similar. The current study confirmed the good action of Fluoroquinolones on preformed biofilms and even the superiority of this activity to that of vancomycin. This result is consistent with the results of Toshihiro and al. [13] which demonstrated better activity of Fluoroquinolones compared to betalactamines and Amikacin on biofilms of *Pseudomonas aeruginosa*, as well as the study of Tanaka.G and al. [13] which demonstrates the superiority of the effect of Fluoroquinolones on biofilms of *Pseudomonas aeruginosa* over betalactamines, the studies of Wafi Siala and al.[15] and J.Bauer and al.[5] also affirm the very good activity of Delafloxacin on the biofilms of *Staphylococcus aureus*. The current study demonstrated a significant anti-biofilm action of Vancomycin in accordance with the results of E.prestrel and al.[8], and contrary to the results of R.C.Evans [2].

Conclusion

The results of our study demonstrate the effectiveness of both Vancomycin and Fluoroquinolones (Ofloxacin and Levofloxacin) and the superiority of the latter on in vitro preformed biofilms of *Staphylococcus sp* at concentrations like those used intraperitoneally.

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