Retrieval of microorganisms from explanted device biofilm: efficacy of three elution methods

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Introduction & Aim

Several methods are available for dislodging microorganisms organized in biofilm and providing quantitative cultures for the microbiological diagnosis of device related infections. In last years, sonication [1] and elution with dithiothreitol (DTT) [2] or Na-mercaptoethanesulfonate (MESNA) [3] have been proposed to treat the surface of explanted devices but comparative studies addressing their efficacy in retrieving microorganisms are scarce.

Central venous catheters (CVC) are frequently colonized by microorganisms causing biofilm formation on catheter surface and lumens and can be used as a convenient model to implement a comparative evaluation of different retrieval methods.

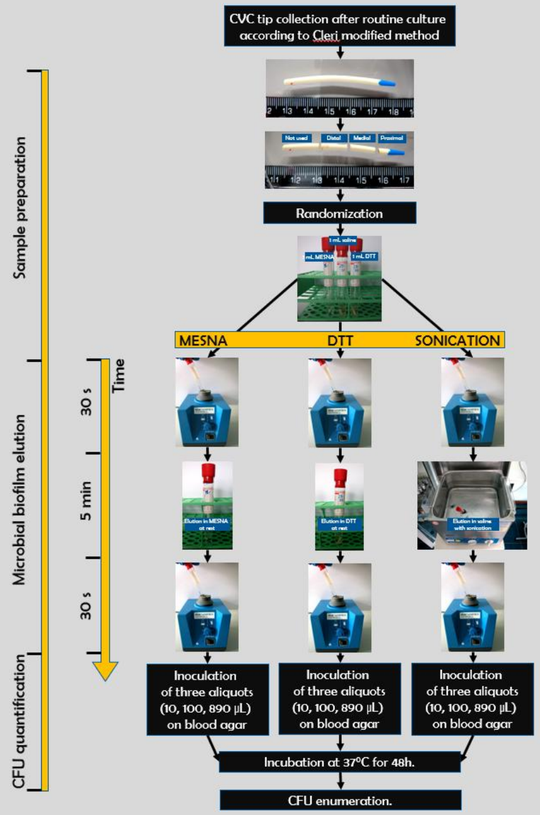
This study aimed at comparing the efficacy of three different eluting protocols based on DTT, MESNA and sonication in retrieving and culturing microorganisms from CVCs removed from patients.

Materials and Methods

A total of 338 CVC tips were collected immediately after routine culture and aseptically sectioned into three segments. Each segment (1 cm long) was randomly assigned to one of the following protocols:

- 30s vortexing, 5min elution, 30s vortexing in 1mL of 1g/L DTT (adapted from [2]);
- 30s vortexing, 5min elution, 30s vortexing in 1mL of 1g/L MESNA (adapted from [3]);
- 30s vortexing, 5min sonication, 30s vortexing in 1mL of sterile saline (adapted from [1]).

Three aliquots (10 µL, 100 µL, and 890 µL) per each eluted suspensions were inoculated onto blood agar plates. Microbial growth and colony count (CFUs) were checked after 24-48h of incubation at 37°C. Retrieval efficacy was assessed on CVC showing at least one positive culture. CFU number obtained from the same volumes were compared among the three elution methods. Wilcoxon signed-rank test with Holms post-hoc correction was used to compare each pair of groups. Two-sided tests with a significance level of $p<0.05$ were considered.



Results

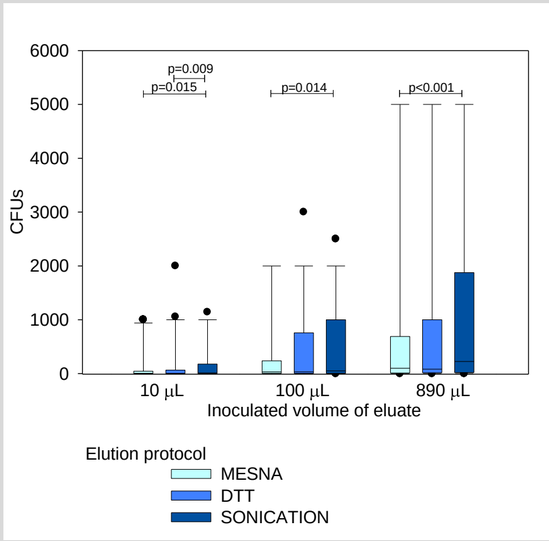
Seventy-six CVCs (22%) showed at least one positive culture. No difference in the isolated species was found among the elution methods. Number of positive cultures and CFUs obtained from the three methods are presented in Table 1. Cultures from 10 µL aliquots showed lower number of positive cultures irrespectively from the elution methods.

Significant differences in retrieved CFU amount were found from:

- 10 µL sonication vs 10 µL DTT (p=0.009),
- 10 µL sonication vs 10 µL MESNA (p=0.015),
- 100 µL sonication vs 100 µL MESNA (p=0.014),
- 890 µL sonication vs 890 µL MESNA (p<0.001).

Table 1: results summary

Cultured Volume	Elution Method	No. of positive culture	CFUs Median [interquartile range]
10 µL	DTT	54	3 [0;59]
	MESNA	54	5 [0;45]
	Sonication	54	13 [0;160]
100 µL	DTT	65	33 [4;715]
	MESNA	67	31 [5;225]
	Sonication	70	50[4;1000]
890 µL	DTT	71	83 [15;1000]
	MESNA	72	100 [13;660]
	Sonication	74	225 [19;1750]



Retrieval efficacy vs elution protocol. Number of CFUs on inoculated plates varies according to the inoculated volume of eluate (10 µL, 100 µL, and 890 µL). Moreover, significant differences were found by comparing CFUs number from different protocols.

Conclusions

Sonication, DTT and MESNA elution could be considered for retrieving microorganisms organized in biofilm on explanted devices providing that an aliquots ≥100 µL of eluate is cultured. Differences in retrieval efficacy among different methods should be considered to properly define specific CFU thresholds for clinical significance of positive cultures.

References

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Acknowledgments

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