MHA MEDIHEALTH ACADEMY

Eurasian Journal of
Anesthesiology & Intensive Care

Original Article

doi
10.51271/EAJAIC-0056

Antibacterial effects of levobupivacaine against *Staphylococcus aureus* in a patient-controlled epidural analgesia model: an in vitro experimental study

Gülay Akıncı*

Department of Anesthesiology and Reanimation, Faculty of Medicine, Dokuz Eylül University, İzmir, Turkiye

Cite this article: Akıncı G. Antibacterial effects of levobupivacaine against *Staphylococcus aureus* in a patient-controlled epidural analgesia model: an in vitro experimental study. *Eur J Anesthesiol Intens Care*. 2026;3(3):63-67. doi:10.51271/EAJAIC-0056

***Corresponding Author:** Gülay Akıncı, drgulayakinci27@gmail.com

Received: 14/05/2026 ◆ **Accepted**: 15/06/2026 ◆ **Published**: 29/08/2026

ABSTRACT

Aims: Catheter-related infections remain an important complication of epidural analgesia. Local anesthetics may contribute to infection prevention because of their antimicrobial properties. This study aimed to evaluate the antibacterial effects of clinically relevant concentrations of levobupivacaine against *Staphylococcus aureus* in a simulated patient-controlled epidural analgesia (PCEA) model.

Methods: An in vitro experimental PCEA infusion system was established using epidural catheter tubing and 0.2-µm bacterial filters. Four study groups were prepared: 0.125% levobupivacaine+fentanyl, 0.0625% levobupivacaine+fentanyl, fentanyl-only solution, and saline control. Each solution was inoculated with *S. aureus* adjusted to a 0.5 McFarland standard. Solutions were infused continuously for 24 hours. Samples obtained from the filter inlet, outlet, and collection chambers were cultured microbiologically. Scanning electron microscopy was used to evaluate bacterial retention at the filter surface.

Results: The saline control group demonstrated the highest bacterial colony counts, whereas levobupivacaine-containing solutions demonstrated significantly lower bacterial colony counts compared with the saline control group ($p<0.05$). Increasing levobupivacaine concentration was associated with stronger antibacterial activity. No bacterial growth was detected distal to the epidural bacterial filter in any study group. SEM analysis confirmed bacterial accumulation on the proximal surface of the filter membrane.

Conclusion: Levobupivacaine exhibited concentration-dependent antibacterial activity against *S. aureus* in a simulated PCEA model, while epidural bacterial filters effectively prevented bacterial passage under experimental conditions. These findings suggest that levobupivacaine may contribute to infection prevention in epidural analgesia systems; however, further in vivo and clinical studies are required to confirm these findings.

Keywords: Levobupivacaine, epidural analgesia, antibacterial activity, *Staphylococcus aureus*, regional anesthesia, PCEA

INTRODUCTION

Regional anesthesia techniques, including epidural and combined spinal-epidural analgesia, are widely used for perioperative analgesia, obstetric anesthesia, and chronic pain management.[1,2] Although epidural analgesia provides effective pain control and improved postoperative recovery, catheter-related infections remain an important concern.[1,2]

The reported incidence of epidural catheter-related infections varies according to catheter duration, patient comorbidities, and aseptic conditions.[1,2] Common causative organisms include skin flora microorganisms such as *Staphylococcus aureus* and *Staphylococcus epidermidis*.[1,3] Epidural abscesses and meningitis, although rare, may lead to severe neurological complications.[2]

To reduce the risk of epidural catheter contamination, strict aseptic technique and bacterial filtration systems are routinely recommended.[1,2] In addition to mechanical barriers, local anesthetics themselves may exhibit antimicrobial effects.[3] Previous studies have reported antibacterial properties of lidocaine, bupivacaine, ropivacaine, and levobupivacaine against various microorganisms.[3-7]

Levobupivacaine is the S(-)-enantiomer of bupivacaine and is widely used in epidural analgesia because of its favorable safety profile and lower cardiotoxicity compared with racemic bupivacaine.[10,11] However, data regarding its antibacterial properties remain limited and conflicting.[10-12] Most previous studies investigated antimicrobial activity under static laboratory conditions using direct bacterial incubation models.[10-14] To our knowledge, few studies have evaluated the antibacterial activity of levobupivacaine using a clinically simulated patient-controlled epidural analgesia (PCEA) infusion model.

Therefore, the aim of this study was to investigate the antibacterial effects of clinically relevant concentrations of levobupivacaine against *S. aureus* in a simulated PCEA system.

METHODS

Ethics

This in vitro experimental study was approved by the Dokuz Eylül University Non-interventional Clinical Researches Ethics Committee (Date: 01.10.2010, Decision No: 271). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki

Study Groups

The experimental solutions were prepared in 100-ml volumes under aseptic conditions.

Group 1 (n=10) 0.125% levobupivacaine+fentanyl

Group 2 (n=10) 0.0625% levobupivacaine+fentanyl

Group 3 (n=10) Fentanyl only

Group 4 (n=10) Saline control

Each solution was inoculated with 1 ml of *S. aureus* suspension adjusted to 0.5 McFarland standard (1.5×10^8 CFU/ml).

The sample size of ten experiments per group was determined based on feasibility considerations and consistency with previous in vitro antimicrobial studies.

Experimental Setup

The experimental infusion system was prepared under strict aseptic conditions. Epidural catheters were connected to 0.2-µm bacterial filters (Portex®, Smiths Medical, USA). The infusion system was attached to a PCA pump (Abbott APM Epidural PCA Pump, USA). Solutions were continuously infused at 4 ml/h for 24 hours at room temperature (Figure 1).

Samples were obtained from:

- Filter inlet
- Filter outlet
- Collection bottles

All samples were cultured on blood agar plates and incubated aerobically at 37°C for 16–24 hours.

SEM Imaging

Scanning electron microscopy (SEM) was performed using a JEOL-JSM-6060 microscope. Filter samples from group 1 and group 4 were processed under vacuum conditions and coated with gold-palladium prior to imaging.

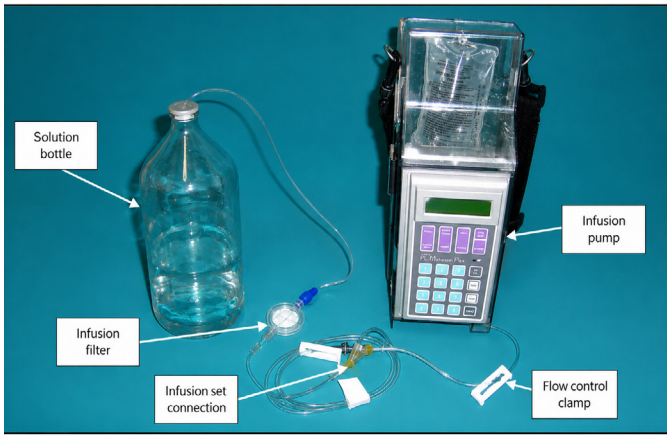

Figure 1. Experimental setup
Schematic representation of the simulated patient-controlled epidural analgesia (PCEA) experimental model.

Statistical Analysis

The data analyses were performed using SPSS version 15.0. Normality of data distribution was assessed using the Shapiro–Wilk test. Since colony count data were not normally distributed, non-parametric statistical methods were used. Data are presented as mean±standard deviation. Comparisons among groups were performed using the Kruskal–Wallis test, followed by Mann–Whitney U tests for pairwise comparisons. A p value<0.05 was considered statistically significant.

RESULTS

Bacterial growth was observed at the filter inlet in all groups. Colony counts at the filter inlet were highest in the saline control group and lowest in the 0.125% levobupivacaine group (Table 1).

Compared with the saline control group, bacterial colony counts were reduced by approximately 56.4% in group 1, 41.3% in group 2, and 27.3% in group 3 (Table 1). Overall group differences were statistically significant (Kruskal–Wallis p<0.0001).

Post-hoc Mann–Whitney U analyses demonstrated statistically significant differences between group 1 and group 4 (p=0.002), group 2 and group 4 (p=0.002), and group 3 and group 4 (p=0.002) (Tables 2–4).

Table 1. Colony counts obtained from filter inlet, outlet, and bottles in all groups (mean±SD) and p values

Groups	Bacterial inoculum (n)	Filter inlet colony count	Filter outlet colony count	Bottle colony count	Overall p value (Kruskal–Wallis)
Group 1	1500000±00	9446±2173	0±00	0±00	
Group 2	1500000±00	12742±2240	0±00	0±00	<0.0001
Group 3	1500000±00	15770±2540	0±00	0±00	
Group 4	1500000±00	21692±2223	0±00	0±00	

Data are presented as mean±SD. Overall group differences were assessed using the Kruskal–Wallis test (p<0.0001). Post-hoc Mann–Whitney U analyses demonstrated significant differences between group 1 versus group 4 (p=0.002), group 2 versus group 4 (p=0.002), group 3 versus group 4 (p=0.002), group 1 versus group 2 (p=0.019), and group 1 versus group 3 (p=0.0001). SD: Standard deviation

The comparison between group 1 and group 2 showed a statistically significant difference (p=0.019), as did the comparison between group 1 and group 3 (p=0.0001) (Tables 5 and 6). The overall comparison among groups 1, 2, and 3 also demonstrated a statistically significant difference (p=0.013) (Table 7).

Table 2. Comparison of bacterial colony counts between group 1 and group 4

Groups	Bacterial inoculum	Filter colony count	Bottle colony	p
Group 1	1500000±00	9446±2173	0±00	0.002
Group 4	1500000±00	21692±2223	0±00	

Table 3. Comparison of group 2 and group 4 colony counts

Groups	Bacterial inoculum	Filter colony count	Bottle colony	p
Group 2	1500000±00	12742±2240	0±00	0.002
Group 4	1500000±00	21692±2223	0±00	

Table 4. Comparison of group 3 and group 4 colony counts

Groups	Bacterial inoculum	Filter colony count	Bottle colony	p
Group 3	1500000±00	15770±2540	0±00	0.002
Group 4	1500000±00	21692±2223	0±00	

Table 5. Comparison of group 1 and group 2 colony counts

Groups	Bacterial inoculum	Filter colony count	Bottle colony	p
Group 1	1500000±00	9446±2173	0±00	0.019
Group 2	1500000±00	12742±2240	0±00	

Table 6. Comparison of group 1 and group 3 colony counts

Groups	Bacterial inoculum	Filter colony count	Bottle colony	p
Group 1	1500000±00	9446±2173	0±00	0.0001
Group 3	1500000±00	15770±2540	0±00	

Table 7. Comparison of group 1, Group 2, and group 3 colony counts

Groups	Bacterial inoculum	Filter colony count	Bottle colony	p
Group 1	1500000±00	9446±2173	0±00	0.013
Group 2	1500000±00	12742±2240	0±00	
Group 3	1500000±00	15770±2540	0±00	

No bacterial growth was detected at the filter outlet or in the collection bottles in any study group (Table 1). SEM imaging demonstrated bacterial accumulation at the filter surface and confirmed the absence of bacterial passage through the filter membrane (Figures 2–4).

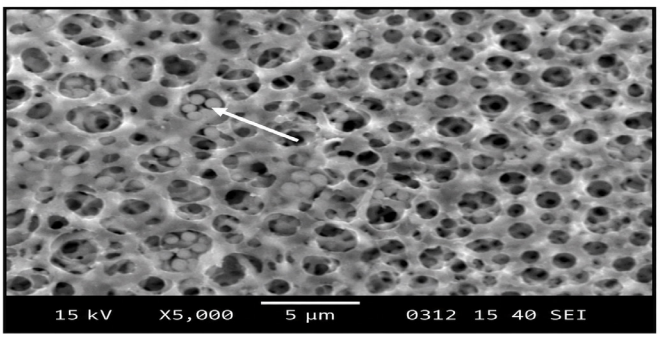

Figure 2. Scanning electron microscopy image of the inlet side of the filter inoculated with *Staphylococcus aureus* in group 1 at a magnification of X5,000. Scanning electron microscopy image demonstrating *Staphylococcus aureus* accumulation on the proximal surface of the epidural bacterial filter membrane in group 1 (×5,000 magnification).

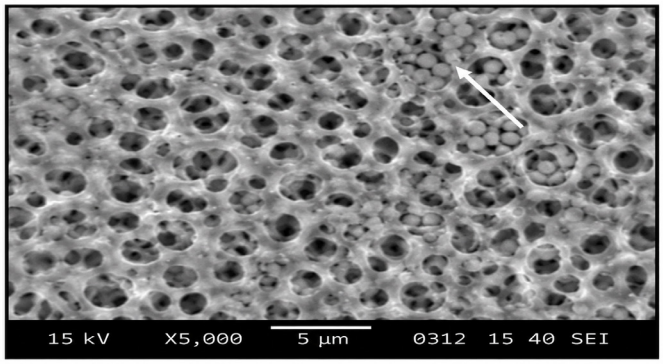

Figure 3. Scanning electron microscopy image of the inlet side of the filter inoculated with *Staphylococcus aureus* in group 4 at a magnification of X5,000. Scanning electron microscopy image demonstrating *Staphylococcus aureus* accumulation on the proximal surface of the epidural bacterial filter membrane in group 4 (×5,000 magnification).

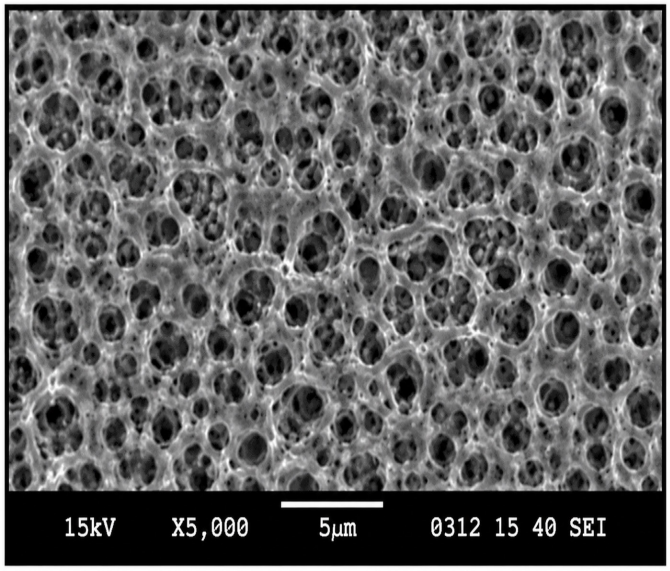

Figure 4. Scanning electron microscopy image of the outlet side of the filter inoculated with *Staphylococcus aureus* at a magnification of X5,000.
Scanning electron microscopy image of the distal surface of the epidural bacterial filter membrane, demonstrating no bacterial passage (×5,000 magnification).

DISCUSSION

The present in vitro experimental study demonstrated that levobupivacaine has a concentration dependent antibacterial effect against *S. aureus* in a simulated PCEA model. The reduction in colony counts was greatest in the 0.125% levobupivacaine group, followed by the 0.0625% levobupivacaine group and fentanyl-only group, while the saline control group had the highest bacterial growth. In addition, no bacterial growth was observed beyond the epidural bacterial filter, suggesting complete bacterial retention under the experimental conditions of this study. These findings are consistent with the original microbiological results and SEM observations of the thesis. Catheter-related infection remains an important safety issue in regional anesthesia, although serious neuraxial infections are rare. Recent regional anesthesia safety recommendations emphasize that contamination may occur through skin flora migration along the catheter, contamination of injected solutions, or breaks in aseptic technique during catheter manipulation.[1,2] Therefore, any pharmacological or mechanical factor that reduces bacterial contamination may have clinical relevance. The antibacterial activity of local anesthetics has been recognized for decades, but recent studies have renewed interest in this topic, particularly because of catheter-associated infections and biofilm formation.[3] Experimental investigations have demonstrated antimicrobial

effects of local anesthetics against clinically important pathogens including *S. aureus*, *S. epidermidis*, and multidrug-resistant bacteria.[4,5] Proposed mechanisms include disruption of bacterial cell membrane integrity, altered membrane permeability, inhibition of membrane-associated enzymatic activity, and interference with cellular metabolic pathways.[6,7]

Recent evidence also suggests that local anesthetics may inhibit biofilm formation. Faustova et al.[8] demonstrated that local anesthetics affected both planktonic forms and biofilm formation capacity of clinical *S. aureus* strains. This observation is clinically important because biofilm formation on epidural catheters contributes to bacterial persistence, resistance to antimicrobial therapy, and chronic catheter colonization.[9]

Our findings are consistent with previous studies reporting antibacterial effects of levobupivacaine against gram-positive microorganisms. Earlier investigations by Hodson et al.[10] demonstrated that levobupivacaine inhibited the growth of *S. aureus* and *S. epidermidis* at clinically relevant concentrations. Guillier et al.[11] also reported that levobupivacaine-containing epidural solutions did not increase bacterial proliferation risk during regional anesthesia applications. However, some studies have reported conflicting findings regarding the antimicrobial potency of levobupivacaine.[12] These discrepancies may be explained by methodological differences including incubation conditions, bacterial inoculum density, exposure duration, and microbiological techniques.

The concentration-dependent effect observed in our study is clinically important. Lower concentrations of local anesthetics are commonly preferred in obstetric and postoperative epidural analgesia to minimize motor blockade and hemodynamic instability. However, our results suggest that dilute concentrations may provide less antibacterial protection than higher concentrations. Similar concentration-dependent antibacterial effects have also been demonstrated for lidocaine and bupivacaine.[13,14]

Compared with the saline control group, bacterial colony counts were reduced by approximately 56.4% in the 0.125% levobupivacaine group, 41.3% in the 0.0625% levobupivacaine group, and 27.3% in the fentanyl-only group. These findings provide a quantitative estimate of the antibacterial activity observed in the present study and further support a concentration-dependent effect of levobupivacaine.

More recent studies further support these findings. Lee et al.[5] reported antibacterial activity of several local anesthetics against multidrug-resistant organisms, with bupivacaine demonstrating strong antimicrobial effects in vitro. Likewise, Heller et al.[4] showed that lidocaine significantly inhibited the growth of *S. Aureus*, MRSA, and *S. epidermidis*. Collectively, these findings support the hypothesis that local anesthetics may contribute to infection prevention during regional anesthesia practice.

One of the major strengths of the present study is the use of a clinically simulated PCEA infusion model rather than a static incubation design. Most previous studies evaluated bacterial growth after direct exposure of microorganisms to local anesthetic solutions under laboratory conditions.[10-14] In contrast, our model included continuous infusion, epidural catheter tubing, bacterial filtration, and prolonged exposure duration, thereby reproducing conditions closer to routine epidural analgesia practice. This methodological approach increases the translational relevance of our findings.

Another important finding was the complete absence of bacterial growth after the epidural bacterial filter. No bacterial colonies were identified in cultures obtained from the filter exit or collection bottles. These findings confirm the effectiveness of 0.2-µm epidural bacterial filters as an additional mechanical barrier against contamination. Recent infection-control literature continues to support the importance of maintaining closed epidural systems and minimizing contamination during catheter manipulation.[1,2]

SEM imaging provided additional confirmation of bacterial retention at the filter surface and strengthened the microbiological findings of the study. The use of SEM imaging constitutes another strength because ultrastructural visualization improves the reliability of microbiological observations and directly demonstrates bacterial entrapment within the filtration system.

Limitations

Several limitations should be acknowledged. First, this was an in vitro study and cannot fully reproduce patient-related variables such as immune response, protein binding, tissue interaction, catheter dwell time, and repeated clinical manipulation. Second, only *S. aureus* was evaluated; future studies should include *S. epidermidis*, methicillin-resistant *S. aureus* (MRSA), gram-negative bacteria, and fungal pathogens. Third, molecular mechanisms responsible for the antibacterial effects were not directly investigated. Therefore, membrane disruption and enzymatic inhibition should be regarded as potential mechanisms suggested by previous studies rather than mechanisms demonstrated in the present study. Fourth, a levobupivacaine-only group was not included. Consequently, the independent antibacterial contribution of levobupivacaine could not be completely separated from any potential antimicrobial effect of fentanyl. Fifth, the infusion system was operated at room temperature rather than physiological temperature (37°C), which may have influenced bacterial growth kinetics and solution stability. Sixth, SEM analysis was performed only in groups 1 and 4; inclusion of the fentanyl-only group could have provided additional comparative information. Finally, biofilm biomass and biofilm-related gene expression analyses were not performed.

CONCLUSION

As a result, levobupivacaine demonstrated concentration-dependent antibacterial activity against *S. aureus* in a clinically simulated PCEA model. Epidural bacterial filters effectively prevented bacterial passage under the experimental conditions of the study. These findings suggest that levobupivacaine may contribute to infection prevention during epidural analgesia; however, further in vivo and clinical studies are required before definitive clinical conclusions can be drawn.

ETHICAL DECLARATIONS

Ethics Committee Approval

This in vitro experimental study was approved by the Dokuz Eylül University Non-interventional Clinical Researches Ethics Committee (Date: 01.10.2010, Decision No: 271).

Informed Consent

As this study was carried out on bacteria in a laboratory setting, written consent was not required.

Peer Review Process

This manuscript was subject to external peer review.

Conflict of Interest

The authors declare no conflicts of interest related to this study.

Financial Disclosure

The authors received no financial support for the conduct or publication of this research.

Author Contributions

The author is solely responsible for the entirety of conception, execution, analysis, and writing of the manuscript.

REFERENCES

1. Azi LMTA, Fonseca NM, Linard LG. SBA 2020: regional anesthesia safety recommendations update. *Braz J Anesthesiol*. 2020;70(4):398-418. doi: 10.1016/j.bjan.2020.02.005
2. Provenzano DA, Hanes M, Hunt C, et al. ASRA Pain Medicine consensus practice infection control guidelines for regional anesthesia and pain medicine. *Reg Anesth Pain Med*. 2025. doi: 10.1136/rapm-2024-105651
3. Johnson SM, Saint John BE, Dine AP. Local anesthetics as antimicrobial agents: a review. *Surg Infect (Larchmt)*. 2008;9(2):205-213. doi: 10.1089/sur.2007.036
4. Heller S, Seemann RJ, Burgkart R, Obermeier A, Locher H. Lidocaine shows significant antimicrobial effects against *Staphylococcus aureus*, MRSA, and *Staphylococcus epidermidis*. *Biomedicines*. 2025;13(1):106. doi: 10.3390/biomedicines13010106
5. Lee JS, Moon J, Lee JY, et al. Antibacterial activity of local anesthetics against multidrug-resistant bacteria. *Microorganisms*. 2025;17(6):4774-4778. doi: 10.62347/GKXF8390
6. Sakuragi T, Ishino H, Dan K. Bactericidal activity of clinically used local anes-thetics on *Staphylococcus aureus*. *Reg Anesth*. 1996;21(3):239–242. doi: 10.1136/rapm-00115550-199621030-00011
7. Ohsuka S, Ohta M, Masuda K, Arakawa Y, Kaneda T, Kato N. Lidocaine hydro-chloride and acetylsalicylate kill bacteria by disrupting the bacterial membrane potential in different ways. *Microbiol Immunol*. 1994;38(6):429–434. doi: 10.1111/j.1348-0421.1994.tb01803.x
8. Faustova M, Nazarchuk O, Dmytriiev D, Babina Y, Nazarchuk H, Dudar A. The effect of local anesthetics against planktonic forms and biofilm formation of *Staphylococcus aureus* clinical strains. *Front Microbiol*. 2023;14:1199899. doi: 10.3389/fmicb.2023.1199899
9. Tuon FF, Suss PH, Telles JP, Dantas LR, Borges NH, Ribeiro VST. Antimicrobial treatment and pathogenesis of *Staphylococcus aureus* biofilm-associated infections. *Pathogens*. 2023;12(1):87-102. doi: 10.3390/antibiotics12010087
10. Hodson M, Gajraj R, Scott NB. A comparison of the antibacterial activity of levo-bupivacaine vs. bupivacaine: an in vitro study with bacteria implicated in epidural infection. *Anaesthesia*. 1999;54(7):699–702. doi: 10.1046/j.1365-2044.1999.00742.x
11. Guillier M, Boselli E, Bouvet L, et al. Levobupivacaine hydrochloride and sufentanil have no antimicrobial effect at 25°C in vitro. *Eur J Anaesthesiol*. 2007;24(7):634–639. doi: 10.1017/S0265021507000026
12. Coghlan MW, Davies MJ, Hoyt C, Joyce L, Kilner R, Waters MJ. Antibacterial activity of epidural infusions. *Anaesth Intensive Care*. 2009;37(1):66-69.
13. Aydin ON, Eyigor M, Aydin N. Antimicrobial activity of lidocaine and bupivacaine on wound pathogens. *Anaesthesia*. 2001;18(10):687-694. doi: 10.1046/j.1365-2346.2001.00900.x
14. Feldman JM, Chapin-Robertson K, Turner J. Do agents used for epidural analgesia have antimicrobial properties? *Reg Anesth*. 1994;19(1):43–47.