Clinical Nutrition ESPEN 30 (2019) 107–112

Contents lists available at ScienceDirect

Clinical Nutrition ESPEN

journal homepage: http://www.clinicalnutritionespen.com

Original article

In vitro comparison of efficacy of catheter locks in the treatment of catheter related blood stream infection

Jakub Visek [a, *], Lenka Ryskova [b], Roman Safranek [c], Martina Lasticova [a], Vladimír Blaha [a]

[a] 3rd Department of Internal Medicine – Metabolic Care and Gerontology, University Hospital and Faculty of Medicine in Hradec Kralove, Charles University in Prague, Sokolska 581, 500 05 Hradec Kralove, Czech Republic
[b] Department of Clinical Microbiology, University Hospital and Faculty of Medicine Faculty in Hradec Kralove, Charles University in Prague, Sokolska 581, 500 05 Hradec Kralove, Czech Republic
[c] Nephrology and Dialysis Department, University Hospital and Faculty of Medicine Faculty in Hradec Kralove, Charles University in Prague, Sokolska 581, 500 05 Hradec Kralove, Czech Republic

ARTICLE INFO

Article history:
Received 14 October 2018
Accepted 24 January 2019

Keywords:
Parenteral nutrition
Catheter-related blood stream infection
Antimicrobial lock therapy

SUMMARY

Background & aims: Venous access used for parenteral nutrition (PN) application is extremely important for patients with intestinal failure. Potential loss of venous access might be a catastrophy for the patient. Catheter infections are a serious complication of PN application. Systemic administration of antibiotics as well as local antibiotic locks into the catheter to sterilize the catheter are used to treat catheter infections. However, there is no clear recommendation applying use of antibiotic locks, that would specify the type and concentration of antimicrobial medication. Our objective were to compare the efficacy of different types of antimicrobial lock therapy (especially taurolidine) and their concentrations to eradicate infectious agents.

Methods: Bacterial strains of microorganisms (*Staphylococcus epidermidis*, *Staphylococcus aureus*, methicillin resistant *S. aureus* (MRSA), *Pseudomonas aeruginosa*, multidrug-resistant *P. aeruginosa*, *Candida albicans*) were used. Subsequently, the catheter was exposed to the microbes and then was incubated with a specific lock for 2 or 24 h at 37 °C. We used these locks: ethanol 70%, taurolidine, gentamicine in concentrations 0,5, 1 and 10 mg/ml and vancomycine in concentrations 1, 5, and 10 mg/ml. The number of remaining CFU (colony forming units) was compared after incubation.

Results: 70% ethanol and taurolidine were most effective for all studied microorganisms. Gentamicine was more effective than vancomycine.

Conclusions: The most effective antimicrobial lock solutions to eradicate selected pathogenic agents were ethanol and taurolidine. Use of antibiotics is often effective after many hours of treatment and there is a risk of inadequate therapy.

1. Introduction

Parenteral nutrition is mostly used to provide nutrition to patients with gut failure [1]. Administration of parenteral nutrition is dependent on the venous access. Proper care of the venous catheter and adequate treatment of complications of its use is essential, especially in patients who depend on long-term home parenteral nutrition, because it affects patient's survival [2]. For home parenteral nutrition, tunnelized venous catheters (eg. Hickman's, Broviack's) and venous port catheters are usually used [3]. As patients' survival improves, availability of venous access is a frequent limitation and their absence might be an indication for gut transplantation [4]. One of the most common complications of the use of catheters is a catheter infection (CRBSI – catheter-related blood stream infection). Data about the incidence of these infections differ in different centers; however, reported median is about 0,82 episodes of CRBSI per 1000 days with a catheter [5]. The most common pathogens associated with CRBSI are *Staphylococcus epidermidis* and *Staphylococcus aureus*. Other common pathogens are *Pseudomonas aeruginosa* and *Candida albicans*. There is an

* Corresponding author.
E-mail addresses: visekjak@fnhk.cz (J. Visek), ryskoval@lfhk.cuni.cz (L. Ryskova), roman.safranek@fnhk.cz (R. Safranek), martina.lasticova@fnhk.cz (M. Lasticova), blaha@lfhk.cuni.cz (V. Blaha).

https://doi.org/10.1016/j.clnesp.2019.01.010
2405-4577/

increasing number of infections caused by strains resistant to antibiotics, like methicillin resistant *S. aureus* (MRSA), and multidrug-resistant *P. aeruginosa* (MR PSAE) [6]. Most frequently, infection is endo or exoluminal, when catheter is contamined from the skin or the end of the catheter is contamined while connecting a bag with parenteral nutrition [7]. Catheter is kept in situ while treating CRBSI if it's possible, since insertion of a new catheter can be problematic because of absence of venous access. Systemic antibiotics can be used for treatment, but they often don't reach lumen of the catheter. Another option is the use of antimicrobial lock therapy (ALT), when antimicrobial agent is administered in a high concentration directly into the catheter [8]. In this case, it gets into the biofilm and eradicates infectious agent. Dwell times for antibiotic lock solutions should generally not exceed 48 h before reinstallation of lock. Antibiotic lock therapy is usually used for 10–14 days [8]. Substances used for catheter disinfection, like ethanol or taurolidine, can also be used as ALT. In case of a serious infection, catheter must be removed. Taurolodine is one of the latest ALTs. It is a modified amino acid taurine with a broad effect on both gram-negative and gram-positive microorganisms, including polyresistant bacteria. Its mechanism of action is the irreversible binding of its hydroxymethyl group to the cell wall and the ability of the bacteria to adhere. Taurolidine thus also prevents the formation of biofilm inside the catheter [9]. After administration, it is metabolized in taurine, carbon dioxide and water in the human body. No resistance to taurolidine has yet been reported. In a number of studies, its preventive use has proven to be effective and a reduction in the number of catheter infections has been achieved [10]. So far, however, there are not enough studies, which compare the effectiveness of taurolidine with other antibiotics in different concentrations and also in the effect on polyresistant microbes. There is also no clear recommendation on the use of different antimicrobial lock therapy. Our goal was to compare effectivity of different types of antimicrobial lock therapy and their concentrations on infectious agents eradication (see Figs. 1–6).

2. Materials and methods

We used modified Andris' method [11] to evaluate antimicrobial effectivity of tested substances. The difference in methodology was based on the use of different evaluation times and the use of different types of antibiotics and their concentrations.

Bacterial strains of *Staphylococcus apidermidis*, *S. aureus*, MRSA, *P. aeruginosa* and multidrug-resistant *P. aeruginosa* (defined as resistance to all agents in at least three out of four classes: fluoroquionolones, aminoglycosides, carbapenems, antipseudomonal penicillins/cephalosporins), *C. albicans* were used. Before each assay the strains were grown aerobically for 18–24 h at 36 °C in BHI broth (Brain-Heart Bouillon, BioMerieux). An aliquot of these cultures (100 μl) was aseptically transferred to fresh BHI broth and grown at 36 °C to reach the middle of the logarithmic phase (optical density [OD], ≈0,6) corresponding to 10^8 CFU (colony forming units/ml. *C. albicans* was grown in Sabouraud broth (BioMerieux)) under the same conditions to reach 10^7 CFU/ml. The turbidimetric method was used for the measurement of optical density.

Tested ALTs were: ethanol 70% (prepared by our hospital pharmacists), taurolidin (Tauro-lock Hep_{100}, (cyclo)-taurolidine, citrate 4% and heparin-mucosa, 100 IU/ml, Tauro-Implant, GmbH, Germany), gentamicin (Gentamicin B. Braun, Germany) in three concentrations prepared by dissolution of the powder, 0,5, 0,1 and 10 mg/ml, vancomycin (Vancomycin Kabi, Fresenius Kabi, s.r.o., Prague, Czech republic), also in three concentrations prepared by dissolution of the powder, 1, 5 and 10 mg/ml. Individual ALTs were tested only on those microbes, in which susceptibility to a given antimicrobial agent was expected. Saline was used as a control solution.

Segments of Broviac catheter (1 cm) were immersed into the human plasma at the temperature of 37 °C during the night. Afterwards, catheter was incubated in TSB (tryptose-soy broth, Bio-Rad) which was inoculated by a strain, at 37 °C for 18–24 h. Then, segments were rinsed thrice in phosphate-buffered saline and incubated in the testing solution for 2 and 24 h at 37 °C. After incubation, segments were ten times washed in phosphate-buffered saline and placed into the TSB and sonicated for 3 min. The next step was counting of survived microbial cells after serial dilutions of a sonicates (series of 6 tenfold dilution in phosphate-buffered saline for each tested concentration and a strain). Each dilution in duplicate (100 μl) was streaked on agar plates (Mueller Hinton 2 agar, Sabouraud agar, BioMerieux). After 24 h of incubation of bacteria and 48 h of yeasts at 37 °C, the number of colonies was counted and recorded for analysis. Antimicrobial effect of individual substances was evaluated by comparison of the number of CFUs after the tested culture was affected by ALT, with the number of CFUs in the control samples (without ALT). All tests were performed in 2 independent experiments.

2.1. Statistical analysis

The statistics were calculated using the software STATISTICA 12. The significance of differences between study groups was determined with ANOVA test. P value of $\leq$0,05 was considered significant. Statistically significant differences when compared the other solutions to the control solution are marked * in Table 1 (Scheffe post–hoc test).

3. Results

Summary results are shown in Table 1. Decrease of CFUs after administration of individual ALTs in different microorganisms is stated. Results are shown in graphs for individual microorganisms.

For *S. epidermidis*, after 2 h there was a decrease in the number of CFUs in 70% ethanol, taurolidine, gentamicine in concentration 1 and 10 mg/ml compared to control, which was statistically significant. After 24 h, there was a statistically significant decrease in the number of CFUs compared to controls in 70% ethanol, taurolidine, gentamicine in all concentrations.

For *S. aureus*, after 2 h exposure number of CFUs decreased statistically significantly in 70% ethanol, taurolidine, gentamicine in concentration 1 and 10 mg/ml compared to controls ($p<0,05$). After 24 h there was a statistically significant decrease in the number of CFUs in all tested ALTs ($p<0,05$).

For *S. aureus – MRSA*, 2-h exposure caused statistically significant decrease of the number of CFUs in 70% ethanol, taurolidine and gentamicine in all concentrations compared to control. After 24 h there was a statistically significant decrease of CFUs in 70% ethanol, taurolidine, gentamicine in all concentrations and vancomycine in concentration 5 and 10 mg/ml ($p < 0,05$).

For *P. aeruginosa*, 2-h as well as 24-h exposure led to a statistically significant decrease in the number of CFUs in all studied ALTs ($p < 0,05$). Vancomycine was not tested.

Results for *multidrug-resistant P. aeruginosa* were similar to naive Pseudomonas, except for gentamicine after 2-h exposure – only gentamicine in concentration 1 and 10 mg/ml led to a statistically significant decrease in the number of CFUs compared to control. Vancomycine was not tested.

70% ethanol and taurolidine were both effective after 2 h exposure in decreasing of CFUs of *C. albicans*. Results were statistically significant ($p < 0,05$). 24-hour test was not performed

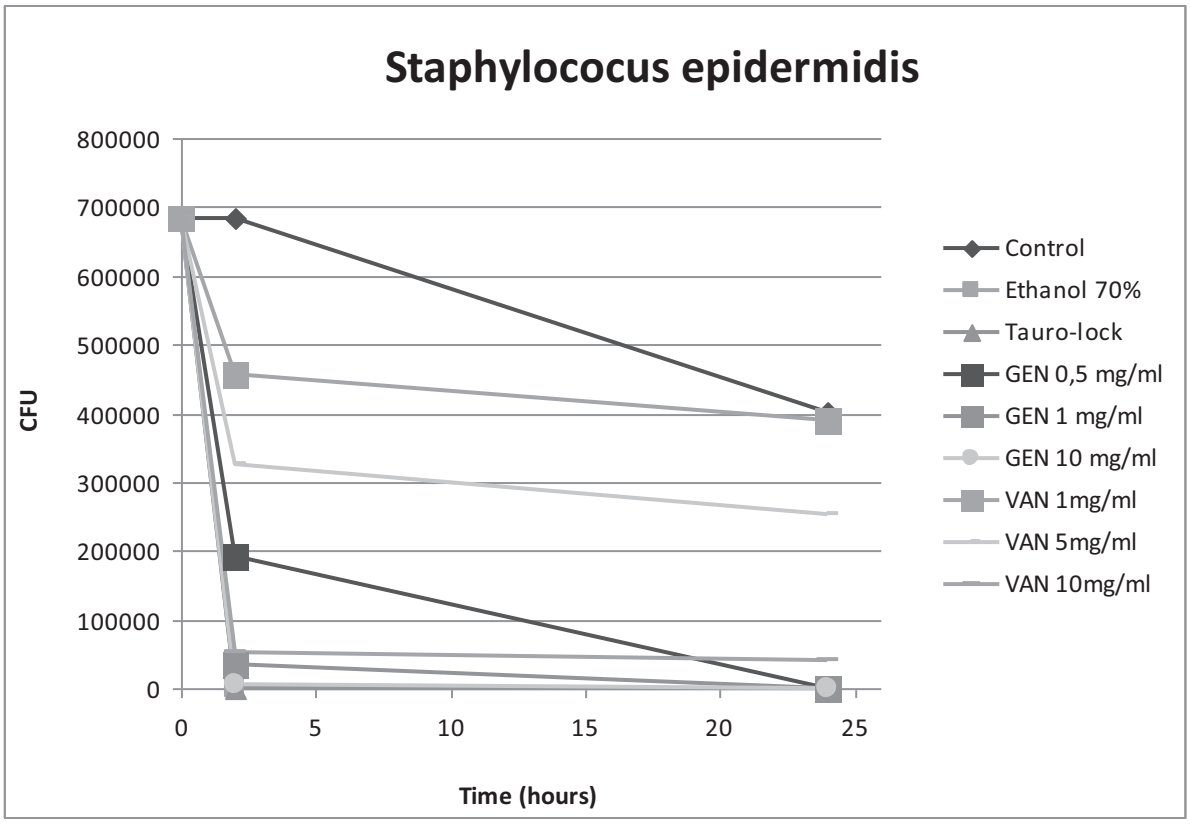

Fig. 1. Decrease of number of CFUs of *Staphylococcus epidermidis* in time after administration of individual ALTs.

because of the proved effectiveness of a 2 h exposure. Vancomycine and gentamicine were not tested.

4. Discussion

Treatment of catheter infections by using of ALT is generally recommended [8]. As we have shown, ALTs are an effective treatment modality in catheter disinfection (sanation) as well as in the treatment of catheter infections. Use of ALTs alone in the treatment of CRBSI is not recommended. Current guidelines recommend combination of ALTs with a systemic antibiotic therapy [8]. Apart from that, clear recommendations regarding the type of a lock and of its concentration in the case of an antibiotic are still lacking. As our results show, appropriately selected ALT can eliminate an infectious agent in 2 h after application, therefore it can be an effective tool in the treatment of CRBSI.

Use of ethanol and taurolidine has shown best results. If an antibiotic was used, its effect was dependent on its type and concentration. Common practice is changing the type of an antibiotic in the lock during the treatment and targeting the treatment after the infectious agent was identified. In this case, there is a risk of a

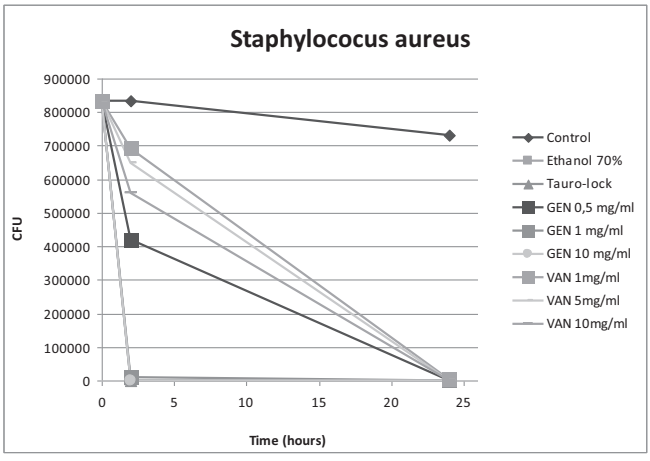

Fig. 2. Decrease of number of CFUs of *Staphylococcus aureus* in time, after administration of individual ALTs.

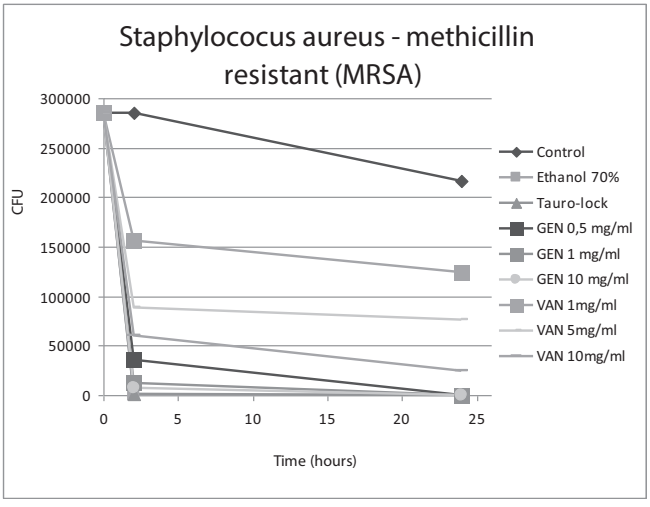

Fig. 3. Decrease of number of CFUs of *methicillin-resistant Staphylococcus aureus – MRSA* in time, after administration of individual ALTs.

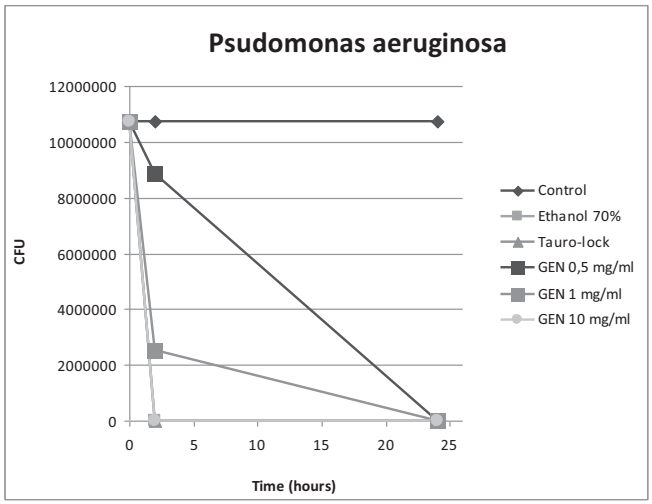

Fig. 4. Decrease of number of CFUs of *Pseudomonas aeruginosa* in time, after administration of individual ALTs.

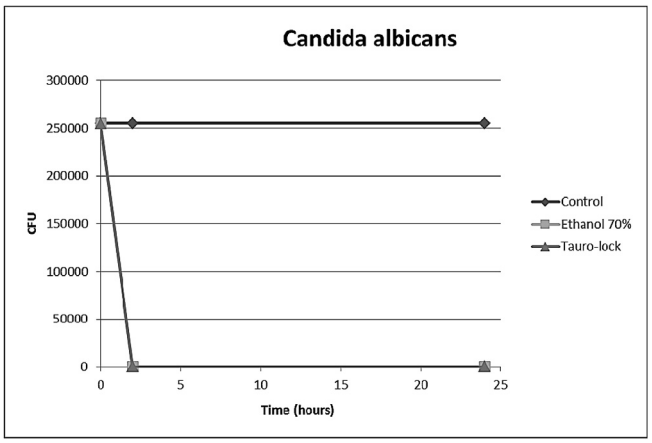

Fig. 6. Decrease of number of CFUs of *Candida albicans* in time after administration of individual ALTs.

primarily inadequately chosen ALT regarding its sensitivity and a delay in the treatment of the infection. Antibiotic resistance development or a secondary mycotic infection is another danger. Some authors report about 8% risk of secondary candidemia development. In a case of a primary enterococcal infection treated by a combination of vancomycine and gentamicine, secondary candidemia developed in 42% cases of the treatment of CRBSI [12], which is an alarming proportion. When taurolidine or ethanol are used, the risk decreases due to their proven efficacy against mycotic agents.

Vancomycine is recommended for empirical treatment of gram-positive bacilli in health care settings because of increased prevalence of MRSA [8]. Nevertheless, its lower efficacy is known in the local treatment of CRBSI, and, therefore its concentration at least 1000x of minimal inhibitory concentration (MIC) in the ALT is recommended, compared to other antibiotics, where it is usually 100x of MIC [13]. Even though these rules were obeyed, its effectivity to Gram negative flora was proved to be lower compared to gentamicine, which was chosen as empirical coverage for gram-negative bacili according to recommendations [8]. One of the possible explanations is a worse ability to enter the biofilm, despite it is an effective bactericidal antibiotic [14]. Microorganisms commonly create a microbial biofilm on an inert surface of the intravascular catheters and other implants, which represents a form of bacterial survival, where bacteria are resistant to antibiotics. Biofilm may slow the distribution of an antibiotic via charge interaction, higher viscosity of the matrix, and possible adsorption to proteins and their inactivation prior to reaching microorganisms [15]. The exact mechanism of this phenomenon is not known. Similar results were observed in clinical studies, in which vancomycine was effective in the treatment of CRBSI only in 37,5%, although its adequate concentration was used [16]. Better results were obtained, if combination of vancomycine with another antibiotic was used [17]. However, vancomycine effectivity is certainly dependent on its concentration.

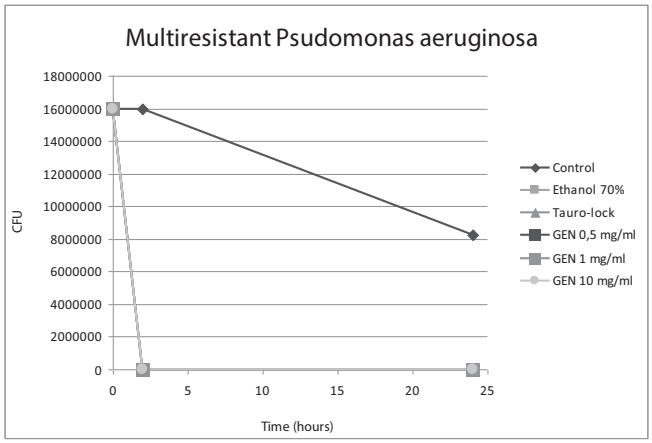

Fig. 5. Decrease of number of CFUs of *multidrug-resistant Pseudomonas aeruginosa* in time, after administration of individual ALTs.

As our results show, ethanol is very effective in microorganisms eradication, but it can have a number of side effects. The most important advantages of its use are a broad antimicrobial effect, low price and also that there is not known that there would be a resistance to its effect. Ethanol can also be used to dissolve deposits of lipids in the catheters, which can formate during parenteral nutrition administration. On the other hand, there are several adverse effects associated with ethanol use. In the literature, cases of disseminated intravascular coagulation, deep venous thrombosis, catheter surface damage (especially polyuretane), were reported. Ethanol affects erythrocyte elasticity, which might increase the risk of hemolysis [18]. Some studies demonstrated promotion of biofilm formation by *Staphylococcus aureus* and epidermidis or increase of resistance to antibiotics by excretory pump stimulation (antibiotic efflux pump) [19]. Use of ethanol might potentially cause a disulfiram effect, if there is a coincidence with metronidazole treatment [18]. Occasionally, other adverse reactions to alcohol can be observed after the catheter flushing, like weakness, palpitation or headache. Particularly in children these reactions are undesirable and it is necessary to consider drawing the ethanol lock out from the catheter.

Taurolidine is a derivative of an aminoacid taurin with a broad spectrum of antimicrobial activity, including activity against Gram negative and Gram positive bacteria and fungi. Basis of its antimicrobial activity is an irreversible binding of its hydroxymethyl group to the cell wall, cell proteins and cell toxins. Microorganisms are destroyed 30 min after taurolidine administration. Taurolidine has also a preventive effect against biofilm formation inside the catheter. In a human it is metabolized to taurine, carbon dioxide and water. Until now, serious complications of taurolidine use are not known, neither development of microbial resistance to its effect. There are data showing possible association between the use

Table 1
The table shows average values of CFUs, as a comparison of effectivity of tested ALTs in reduction of CFUs in time compared to control solution (saline) (Gen – gentamycine, VAN – vancomycine). Stastistically significant differences when compared to the control solution are marked * (ANOVA – Scheffé post–hoc test).

	S. epid. 2 h	*S. epid.* 24 h	*S. aureus* 2 h	*S. aureus* 24 h	MRSA 2 h	MRSA 24 h	PSAE 2 h	PSAE 24 h	MR PSAE 2 h	MR PSAE 24 h	*C. albicans* 2 h	*C. albicans* 24 h
Control	685,000	400,500	832,500	732,500	285,000	616,500	10,750,000	10,750,000	16,000,000	16,000,000	25,500	not tested
Ethanol	20*	0*	0*	0*	13*	0*	0*	0*	285*	50*	7,5*	not tested
Taurolidine	0*	25*	0*	0*	1488*	5*	95*	0*	28*	0*	0*	not tested
GEN 0,5 mg/ml	192,250	105*	420,000	60*	36250*	50*	16750*	1450*	8,850,000	0*	not tested	not tested
GEN 1 mg/ml	35750*	0*	11125*	10*	10000*	5*	1250*	0*	2535000*	0*	not tested	not tested
GEN 10 mg/ml	5945*	0*	1175*	0*	7800*	0*	1580*	0*	1020*	0*	not tested	not tested
VAN 1 mg/ml	456,500	390,500	690,000	4400*	156,250	123,600	not tested	not tested	not tested	not tested	not tested	not tested
VAN 5 mg/ml	327,250	254,000	650,000	64*	88750*	76050*	not tested	not tested	not tested	not tested	not tested	not tested
VAN 10 mg/ml	51,500	42,000	560,000	0*	59750*	24750*	not tested	not tested	not tested	not tested	not tested	not tested
Anova test	$p = 0,005$	$p < 0,001$	$p < 0,001$	$p < 0,001$	$p < 0,001$	$p < 0,001$	$p < 0,001$	$p < 0,001$	$p < 0,001$	$p < 0,001$	$p = 0,007$	

of taurolidine and reversible neutropenia and thrombocytopenia [20]. Our results clearly show, that taurolidine has a rapid effect and an extraordinarily broad spectrum of antimicrobial activity.

Our results show high effectivity of ethanol and taurolidine on microbial eradication in vitro. More clinical studies are needed to prove that treatment with taurolidine and ethanol is more effective than antibiotic locks in clinical practice.

5. Conclusion

Taurolidine and 70% ethanol seem to be the most effective ALTs in eradication of selected pathogenic agents in-vitro. Use of antibiotics is often effective after many hours of treatment and there is a risk of inadequate therapy. More data from clinical studies are needed to prove that treatment with taurolidine or ethanol is more effective than antibiotic locks in the treatment of catheter-related blood stream infection.

Statement of authorship

Jakub Visek, Roman Safranek, Martina Lasticova equally contributed to the conception and design of the research. Jakub Visek drafted the manuscript. Lenka Ryskova, contributed to the acquisition and analysis of the data. Prof. Vladimir Blaha contributed to the interpretation of the data. All authors critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

Funding sources

This research was supported by Progres Q40.

Conflict of interest

The authors declare that there are no conflicts of interest or any commercial associations regarding the publication of this article.

CRediT authorship contribution statement

Jakub Visek: Conceptualization, Formal analysis, Project administration, Writing - original draft. **Lenka Ryskova:** Investigation, Methodology, Writing - review & editing. **Roman Safranek:** Resources, Writing - review & editing. **Martina Lasticova:** Writing - review & editing. **Vladimír Blaha:** Project administration, Resources, Supervision.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2019.01.010.

References

[1] Bielawska B, Allard JP. Parenteral nutrition and intestinal failure. Nutrients 2017 May 6;9(5) [Internet]. Multidisciplinary Digital Publishing Institute (MDPI) [cited 2018 Dec 31] Available from: http://www.ncbi.nlm.nih.gov/pubmed/28481229.

[2] Dibb M, Lal S. Home parenteral nutrition: vascular access and related complications. Nutr Clin Pract 2017 Dec 12;32(6):769–76 [Internet] John Wiley & Sons, Ltd; [cited 2018 Dec 31] Available from: http://doi.wiley.com/10.1177/0884533617734788.

[3] Pironi L, Arends J, Bozzetti F, Cuerda C, Gillanders L, Jeppesen PB, et al. ESPEN guidelines on chronic intestinal failure in adults. Clin Nutr 2016 Apr 1;35(2):247–307 [Internet]. Elsevier [cited 2018 Dec 31] Available from: http://www.ncbi.nlm.nih.gov/pubmed/26944585.

[4] Middleton SJ, Jamieson NV. The current status of small bowel transplantation in the UK and internationally. Gut 2005 Nov;54(11):1650–7 [Internet]. BMJ Publishing Group [cited 2018 Dec 31]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/16227364.

[5] Dreesen M, Foulon V, Spriet I, Goossens GA, Hiele M, De Pourcq L, et al. Epidemiology of catheter-related infections in adult patients receiving home parenteral nutrition: a systematic review. Clin Nutr 2013 Feb 1;32(1):16–26 [cited 2018 Dec 31] [Internet]. Elsevier; Available from: http://www.ncbi.nlm.nih.gov/pubmed/22959630.

[6] Han Z, Liang SY, Marschall J. Current strategies for the prevention and management of central line-associated bloodstream infections. Infect Drug Resist 2010;3:147–63 [Internet]. Dove Press; [cited 2018 Dec 31]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/21694903.

[7] Gahlot R, Nigam C, Kumar V, Yadav G, Anupurba S. Catheter-related bloodstream infections. Int J Crit Illn Inj Sci 2014 Apr;4(2):162–7 [Internet]. Wolters Kluwer – Medknow Publications [cited 2018 Dec 31]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25024944.

[8] Mermel LA, Allon M, Bouza E, Craven DE, Flynn P, O'Grady NP, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 Update by the Infectious Diseases Society of America. Clin Infect Dis 2009 Jul 1;49(1):1–45 [Internet]. NIH Public Access [cited 2018 Dec 31]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19489710.

[9] Torres-Viera C, Thauvin-Eliopoulos C, Souli M, DeGirolami P, Farris MG, Wennersten CB, et al. Activities of taurolidine in vitro and in experimental enterococcal endocarditis. Antimicrob Agents Chemother 2000 Jun;44(6):1720–4 [Internet]. American Society for Microbiology (ASM) [cited 2018 Dec 31]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10817739.

[10] Al-Amin AH, Sarveswaran J, Wood JM, Burke DA, Donnellan CF. Efficacy of taurolidine on the prevention of catheter-related bloodstream infections in patients on home parenteral nutrition. J Vasc Access 2013 Oct 7;14(4):379–82 [Internet]. SAGE PublicationsSage UK: London, England [cited 2018 Dec 31]. Available from: http://journals.sagepub.com/doi/10.5301/jva.5000168.

[11] Andris DA, Krzywda EA, Edmiston CE, Krepel CJ, Gohr CM. Elimination of intraluminal colonization by antibiotic lock in silicone vascular catheters. Nutrition 1998 May 1;14(5):427–32 [Internet]. Elsevier [cited 2018 Dec 31]. Available from: https://www.sciencedirect.com/science/article/pii/S0899900798000136?via%3Dihub.

[12] Krishnasami Z, Carlton D, Bimbo L, Taylor ME, Balkovetz DF, Barker J, et al. Management of hemodialysis catheter-related bacteremia with an adjunctive antibiotic lock solution. Kidney Int 2002 Mar 1;61(3):1136–42 [Internet]. Elsevier [cited 2018 Dec 31]. Available from: http://linkinghub.elsevier.com/retrieve/pii/S008525381548322X.

[13] Vanholder R, Canaud B, Fluck R, Jadoul M, Labriola L, Marti-Monros A, et al. Catheter-related blood stream infections (CRBSI): a European view. Nephrol Dial Transplant 2010 Jun 1;25(6):1753–6 [Internet]. Oxford University Press [cited 2018 Dec 31]. Available from: https://academic.oup.com/ndt/article-lookup/doi/10.1093/ndt/gfq205.

[14] Antunes ALS, Bonfanti JW, Perez LRR, Pinto CCF, Freitas ALP, de Macedo AJ, et al. High vancomycin resistance among biofilms produced by Staphylococcus species isolated from central venous catheters. Mem Inst Oswaldo Cruz 2011 Feb;106(1):51–5 [Internet]. Fundação Oswaldo Cruz [cited 2018 Dec 31]. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02762011000100008&lng=en&tlng=en.

[15] Justo JA, Bookstaver PB. Antibiotic lock therapy: review of technique and logistical challenges. Infect Drug Resist 2014;7:343–63 [Internet]. Dove Press [cited 2018 Dec 31]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25548523.

[16] Bookstaver PB, Gerrald KR, Moran RR. Clinical outcomes of antimicrobial lock solutions used in a treatment modality: a retrospective case series analysis. Clin Pharmacol 2010;2:123–30 [Internet]. Dove Press [cited 2018 Dec 31]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22291495.

[17] Rose WE, Poppens PT. Impact of biofilm on the in vitro activity of vancomycin alone and in combination with tigecycline and rifampicin against Staphylococcus aureus. J Antimicrob Chemother 2009 Jan 15;63(3):485–8 [Internet]. Oxford University Press [cited 2018 Dec 31]. Available from: https://academic.oup.com/jac/article-lookup/doi/10.1093/jac/dkn513.

[18] Mermel LA, Alang N. Adverse effects associated with ethanol catheter lock solutions: a systematic review. J Antimicrob Chemother 2014 Oct 1;69(10):2611–9 [Internet]. Oxford University Press [cited 2018 Dec 31]. Available from: https://academic.oup.com/jac/article-lookup/doi/10.1093/jac/dku182.

[19] Redelman CV, Maduakolam C, Anderson GG. Alcohol treatment enhances *Staphylococcus aureus* biofilm development. FEMS Immunol Med Microbiol 2012 Dec 1;66(3):411–8 [Internet]. John Wiley & Sons, Ltd (10.1111) [cited 2018 Dec 31]. Available from: https://academic.oup.com/femspd/article-lookup/doi/10.1111/1574-695X.12005.

[20] Sherertz RJ, Boger MS, Collins CA, Mason L, Raad II . Comparative in vitro efficacies of various catheter lock solutions. Antimicrob Agents Chemother 2006 May;50(5):1865–8 [Internet]. American Society for Microbiology (ASM) [cited 2018 Dec 31]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16641463.