PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Comparison of olanexidine versus povidone-iodine for preventing surgical site infection in gastrointestinal surgery: study protocol for a multicenter, single-blind, randomized controlled clinical trial
AUTHORS	Takeuchi, Masashi; Obara, Hideaki; Kawakubo, Hirofumi; Shinoda, Masahiro; Okabayashi, Koji; Mayanagi, Shuhei; Irino, Tomoyuki; Fukuda, Kazumasa; Nakamura, Rieko; Wada, Norihito; Kitago, Minoru; Yagi, Hiroshi; Abe, Yuta; Oshima, Go; Hori, Shutaro; Tsuruta, Masashi; Ishida, Takashi; Yokose, Takahiro; Hirukawa, Kazuya; Isobe, Yoh; Sekimoto, Yasuhito; Harada, Hirohisa; Maeda, Yusuke; Shito, Masaya; Kondo, Takayuki; Sato, Yasunori; Kitagawa, Yuko

VERSION 1 - REVIEW

REVIEWER	Markus W. Büchler Department of General, Visceral and Transplantation Surgery, University Hospital Heidelberg, Germany
REVIEW RETURNED	06-Dec-2018

GENERAL COMMENTS	Thank you for the opportunity to review the clinical trial protocol by Takeuchi et al. entitled 'Comparison of olanexidine versus povidone-iodine for preventing surgical site infection in gastrointestinal surgery: study protocol for a multicenter, single-blind, randomized controlled clinical trial'. The authors describe the protocol for a multicenter randomized clinical trial with two parallel study groups comparing skin disinfection with povidone-iodone (control group) vs. olanexidine. Overall there are several important aspects that should be addressed and I believe that the protocol can be published with major revisions. Unfortunately, the trial has already started recruitment in May or June 2018, therefore our comments will likely have little impact on the future conduct of the trial. This is a recurrent problem with many clinical trial protocols, as frequently trial protocols are submitted for publication only after the trial has already been initiated. We encourage the authors to address the following aspects: 1. Please adapt the protocol to the international SPIRIT guidelines and provide a SPIRIT checklist as supplement. The guidelines can be accessed at www.spirit-statement.org/ 2. Please describe the intervention and control in more detail: a. An important issue is the use of alcohol as an adjunct in the interventional and control group. Alcohol-based skin disinfectant are unanimously recommended by international guidelines (e.g.

	WHO, CDC; https://jamanetwork.com/journals/jamasurgery/fullarticle/2623725; http://apps.who.int/iris/bitstream/handle/10665/250680/9789241549882-eng.pdf;jsessionid=81B101E4C2AE0DBC41AFD136A7FC496C?sequence=1 etc.). As a consequence, there is a widespread but incorrect perception in the health-care community suggesting that evidence for the chlorhexidine–alcohol combination constitutes evidence for chlorhexidine. Please refer to Maiwald et al. Lancet 2014 for more details of this discussion (1). These problems should be addressed in the proposed trial. b. There is substantial evidence, that the mode of application of the skin antiseptic has an effect on surgical site infection rate (SSI) (e.g. Edmiston et al. (2)). Therefore, the mode of application should be the same in both groups and should be outlined in detail. c. Similarly, the duration of application prior to incision influences the antimicrobial effect. Thus, please standardize the application duration between the groups. 3. The trial protocol approved by the local ethic committee is dated October/ 24/2018, ver1.3), however recruitment of the trial seems to have started prior to this date (May or June 2018). Please clarify and provide all previous version of the protocol as supplement. 4. Please provide a table with details of the trial visits. When exactly are the visits performed? What data are collected? Please refer to SPIRIT guidelines for examples. Please also provide a flow-chart of the trial. 5. Please specify when randomization takes place. Ideally, randomization should be conducted on the day of surgery just prior to skin disinfection to exclude bias. 6. Multiple factors have been associated with an enhanced/reduced risk of SSI infections including BMI, diabetes mellitus, smoking, the use of prophylactic antibiotics, the use of wound edge protectors, mode of skin closure etc. Some of these factors (laparoscopic vs. open, wound class) have been accounted for in the current trial, either by means of stratification or by eligibility criteria. Some other factors are collected during the trial, but are not accounted for in the analysis. However, ALL remaining risk/protective factors could act as confounders in the planned trial. Therefore, data on these parameters has to be collected and needs to be accounted for in the analysis of the primary endpoint (SSI rate). We are confused as to what analysis methods the authors want to apply for the primary outcome. In the abstract it says “Pearson’s chi-square test or Fisher’s exact test and Student’s t-test will be used in the statistical analyses”. In the methods section the Mantel-Haenszel test is mentioned. Importantly, all potential confounders need to be considered in the analysis. Why do the authors not use a multivariate binary logistic regression analysis for primary endpoint analysis? 7. The authors claim to perform an outcome-assessor blinded trial. However, they state (page 15, 31 “…Diagnosis of SSI, which is reported by nonblinded investigators, will be verified by chart review, and blinded investigators will verify the diagnosis”). This is NOT sufficient masking of the outcome assessors, as only those patients will be assessed by the blinded investigators that have been “reported” by the unblinded investigators. Much rather, ALL

	patients need to be visited by blinded outcome assessors at fixed postoperative intervals. 8. Please outline in more detail how blinding is maintained. Are operation reports blinded? Is unblinding possible (e.g. in the case of a serious adverse event). Why is data entry not blinded (p19, 41)? This should be implemented. Similarly, data analysts can be blinded. Please specify for each step of the trial who is blinded. In surgical trials the term “single- “or “double-blind” is meaningless and confusing. We recommend: a. Surgeon: unblinded b. Patient: blinded c. Outcome assessor: blinded d. Data entry: blinded e. Data analyst: blinded. 9. Please be more specific in defining your outcome measures (erythema, etc.) The primary outcome is SSI according to the CDC definition. 10. Please be very specific whether you want to analysis ALL (i.e. superficial, deep and organ-space) SSIs as primary endpoints or only superficial and deep SSIs. Given the mode of action of a skin disinfectant it sounds reasonable to assume that only infections by skin pathogens will be reduced. This is supported by antimicrobial analyses of SSIs in gastrointestinal surgery. Thus, most likely, only superficial and deep wound infections will be influenced by the intervention. Why are organ-space infections included in the primary endpoint analysis? Do the authors think that organ-space infections like anastomotic leakage, infected pancreatic fistula etc. (all counting as organ-space infections) are influenced by the choice of skin disinfectant? 11. Similarly, the protocol needs to clarify the statistical analysis populations. This is not trivial in SSI trials. We recommend performing the primary endpoint analysis in the ITT population rather than using the “the full analysis set (FAS)”. Definition of the FAS by the authors (“…took at least one dose of treatment during the study, did not have any serious violation of the study protocol, and had data collected after treatment commencement”) is vague and not adapted to the current trial. What is meant with “took at least one dose of treatment”? Furthermore, the FAS introduces bias as patients can be excluded at the discretion of the investigators (“had data collected…, did not have a serious violation of the study protocol…”). We strongly recommend using the ITT population for analysis and the PP and SSI population for sensitivity analysis. Definitions could be as follows: a. Intention-to-treat population comprises all patients randomized and these patients will be analyzed as randomized. However, a number of patients will not be able to undergo visit at postoperative day 30, either due to death, relaparotomy or lost to follow-up. The authors need to specify for each of these cases how the primary endpoint is imputed in these cases. E.g. is death counted as SSI? b. Per-protocol population: Patients are analyzed as treated, not as randomized and only those patients are considered that have finished follow-up at day 30. c. SSI population: all patients randomized that have fulfilled their follow-up at day 30.

	12. The follow-up visits need to be clarified. At what days are patients seen? by whom? and how are assessors blinded? Minor comments: • The CDC SSI definition is well known. There is no need to repeat it in the protocol. Please delete or put it into the supplement. • Exclusion criterion 4: “Receipt of antimicrobial therapy…” please delete “receipt of”. • There are contradictory statements concerning the date of initiation (Abstract. June 2018, methods section: May 2018). Please clarify • Page 11, 21 please replace “useful” with “more effective”. 1. Maiwald M, Assam PN, Chan ES-Y, Dancer SJ. Chlorhexidine’s role in skin antisepsis: questioning the evidence. The Lancet. 2014 Oct 11;384(9951):1344–5. 2. Edmiston CE, Seabrook GR, Johnson CP, Paulson DS, Beausoleil CM. Comparative of a new and innovative 2% chlorhexidine gluconate-impregnated cloth with 4% chlorhexidine gluconate as topical antiseptic for preparation of the skin prior to surgery. Am J Infect Control. 2007 Mar;35(2):89–96. 3. Bruce J, Russell EM, Mollison J, Krukowski ZH. The quality of measurement of surgical wound infection as the basis for monitoring: a systematic review. J Hosp Infect. 2001 Oct;49(2):99–108.

REVIEWER	Olivier MIMOZ University Hospital of Poitiers
REVIEW RETURNED	17-Dec-2018

GENERAL COMMENTS	The authors report a study protocol aimed at comparing two antiseptic solutions (1.5% olanexidine and 10% povidone iodine) to reduce the rate of surgical site infection (SSI) in gastrointestinal surgery. Six hundred patients from 4 Japanese hospitals will be randomised into two groups. The primary endpoint will be the 30-day postoperative SSI rate. Identifying the best antiseptic to prevent SSI is an important step, as the rate of SSI remains high despite improved patients’ care. Unfortunately, the proposed study protocol suffers from several limitations that should be addressed. 1. The study is conducted in four hospitals and includes various gastrointestinal surgeries with different risk level of developing SSIs. No stratification is planned. The methodology used and the data collected are insufficiently developed in the manuscript. Practices may vary from one surgeon and/or anaesthetist to another. Thus, it is impossible to know if the 2 groups will be comparable in terms of risk factors for developing SSI at the end of the study. How imbalances between groups will be taken into account when performing the statistical analyses should be added in the manuscript. 2. No information is given concerning the monitoring of the study in order to know if the protocol is respected by all healthcare providers. 3. Little information is given on the 2 antiseptics used and how they will be applied. In addition, the choice of povidone-iodine in aqueous solution as the reference group appears unjustified in 2018 – A large literature has demonstrated the superiority of alcoholic formulations over aqueous formulations of antiseptics.

	Please find below my comments in details Abstract. 1. Introduction should better describe the issue being studied and how this study could improve clinical practices. 2. What is a Class II surgical wound? 3. No information is provided on the approval of the study by an ethics committee. Strengths and limitations. 1. The second point is neither a strength nor a limitation of the study and should be deleted. 2. At least one sentence on the limits of the study should be added. Introduction. 1. Gastrointestinal surgery is a clean-contaminated surgery where the microorganisms involved in ISS come from the skin and digestive tract. Therefore, skin preparation may prevent only a fraction of the SSIs (those due to skin bacteria). This point should be discussed by the authors. 2. Similarly, the superiority of alcoholic formulations over aqueous formulations of antiseptics is not addressed. 3. WHO guidelines (ref 8) recommend the use of alcoholic chlorhexidine for skin preparation before surgery. 4. Chlorhexidine is less effective against MRSA than against MSSA, but it is not true that chlorhexidine is ineffective against MRSA or VRE. 5. Is olanexidine available outside Japan? 6. Olanexidine is bactericidal and not bacteriostatic 7. Please specify whether olanexidine is used in alcoholic or aqueous formulation and whether the comparator is aqueous of alcoholic povidone iodine – also indicate the concentration of povidone iodine. Methods 1. Please explain what a class 2 surgical wound is 2. More information is needed on how to apply antiseptics (number of applications, modality, time….). Particularly, do you use olanexidine applicators? 3. In addition, more information is needed on other measures applied to prevent SSI before, during and after surgery, as well as how they will be monitored (shower, hair removal, antibiotic prophylaxis, normothermia....). 4. Data collection. All risk factors and prevention methods of SSI should be collected during the perioperative period. These data will be useful to adjust the results in cases of differences between groups. 5. Sample size calculation. The incidence of SSIs with use of aqueous povidone iodine observed in previous studies should be added to support their estimation. 6. Statistical analysis. “one dose of treatment” is not appropriate Fig 1 is a table A figure with the flow chart of the study is missing

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Comment 1: Please adapt the protocol to the international SPIRIT guidelines and provide a SPIRIT checklist as supplement. The guidelines can be accessed at www.spirit-statement.org/

Reply: We have completed the SPIRIT checklist and included the relevant page numbers from our manuscript. We have attached the SPIRIT checklist as a supplement.

Comment 2: Please describe the intervention and control in more detail:

a. An important issue is the use of alcohol as an adjunct in the interventional and control group. Alcohol-based skin disinfectant are unanimously recommended by international guidelines (e.g. WHO, CDC; https://jamanetwork.com/journals/jamasurgery/fullarticle/2623725; http://apps.who.int/iris/bitstream/handle/10665/250680/9789241549882-eng.pdf;jsessionid=81B101E4C2AE0DBC41AFD136A7FC496C?sequence=1 etc.). As a consequence, there is a widespread but incorrect perception in the health-care community suggesting that evidence for the chlorhexidine–alcohol combination constitutes evidence for chlorhexidine. Please refer to Maiwald et al. Lancet 2014 for more details of this discussion (1). These problems should be addressed in the proposed trial.

Reply: Many studies have indicated the antiseptic superiority of chlorhexidine-alcohol compared to povidone-iodine; however, few randomized trials have reported superiority in clean-contaminated gastrointestinal surgery. Furthermore, since inflammability is associated with chlorhexidine-alcohol, povidone-iodine is recommended and typically used for gastrointestinal surgery in Japan. In addition, chlorhexidine-alcohol with concentrations greater than 1% are not commercially available in Japan, although a concentration greater than 2% is recognized as having a bactericidal effect in international guidelines.

Moreover, considering the influence of ethnic differences including intrinsic and extrinsic ethnic factors,[1] this trial is a meaningful examination of surgical site infection (SSI) treatment at least in Japan. Therefore, we think that the selection of a control group is reasonable.

As you noted, in the future, we should also consider conducting another trial that compares olanexidine to an alcohol-based antiseptic agent, if superiority of olanexidine compared to povidone-iodine is proven in the present trial. This information has been included in the Discussion section (page 21, lines 1-9).

b. There is substantial evidence, that the mode of application of the skin antiseptic has an effect on surgical site infection rate (SSI) (e.g. Edmiston et al. (2)). Therefore, the mode of application should be the same in both groups and should be outlined in detail.

Reply: The antiseptic should be applied widely in consideration of the drain site and length of the skin incision. We apply agents on the papilla (in cases of esophageal surgery: the neck) with a cranial limit and to the upper thigh with a caudal limit. After waiting 3 minutes to allow the antiseptics to dry, the operation is started. Both antiseptics are used in the same way. This information has been added in the Methods section (page 11, lines 5-9).

c. Similarly, the duration of application prior to incision influences the antimicrobial effect. Thus, please standardize the application duration between the groups.

Reply: As described earlier, we made the incision after waiting 3 minutes for the antiseptic to dry. This information has been clarified in the Methods section (page 11, lines 5-9).

Comment 3: The trial protocol approved by the local ethic committee is dated October/ 24/2018, ver1.3), however recruitment of the trial seems to have started prior to this date (May or June 2018). Please clarify and provide all previous version of the protocol as supplement.

Reply: The trial protocol first approved by the ethics committee of Keio University School of Medicine was dated March 15, 2018 (version 1.0), followed by version 1.1 dated June 17, 2018. Then, we submitted version 1.2 of the protocol to the ethics committee; however, they requested major revision of protocol. Therefore, the final protocol is version 1.3 dated October 24, 2018. We provided final protocol and primary protocol using the track changes mode in MS Word as supplemental.

Comment 4: Please provide a table with details of the trial visits. When exactly are the visits performed? What data are collected? Please refer to SPIRIT guidelines for examples. Please also provide a flow-chart of the trial.

Reply: Generally, patients are hospitalized 1 to 4 days before surgery. We obtain informed consent and patients' background characteristics after admission. Informed consent for the operation and the clinical trial is routinely obtained on the day before surgery. Thus, randomization is mainly performed on the day before surgery. The investigators will observe the surgical wound site daily during admission. The duration of observation will be 30 days postoperatively. After discharge, participants will undergo outpatient observation at least once if within 30 days postoperatively. This information has been added in the Methods section (page 14, lines 4-9) and Table 2.

Table 2 Flow chart of the trial

Time point	After admission	Before surgery	Surgery	After surgery
Informed consent	□			
Patient's background characteristics	□			
Physical examination	□			
Randomization		□		
Intervention			□	
Observation of the surgical site				□a

□a: From postoperative day 1 to postoperative day 30 (outpatient observation is performed at least once if the discharge is within 30 days postoperatively)

Comment 5: Please specify when randomization takes place. Ideally, randomization should be conducted on the day of surgery just prior to skin disinfection to exclude bias.

Reply: Informed consent for the operation and clinical trial is routinely obtained on the day before surgery. Thus, randomization is mainly performed on the day before surgery. This has been clarified in the Methods (page 14, lines 4-9).

Comment 6: Multiple factors have been associated with an enhanced/reduced risk of SSI infections including BMI, diabetes mellitus, smoking, the use of prophylactic antibiotics, the use of wound edge protectors, mode of skin closure etc. Some of these factors (laparoscopic vs. open, wound class) have been accounted for in the current trial, either by means of stratification or by eligibility criteria. Some other factors are collected during the trial, but are not accounted for in the analysis. However, ALL remaining risk/protective factors could act as confounders in the planned trial. Therefore, data on these parameters has to be collected and needs to be accounted for in the analysis of the primary endpoint (SSI rate). We are confused as to what analysis methods the authors want to apply for the primary outcome. In the abstract it says “Pearson’s chi-square test or Fisher’s exact test and Student’s t-test will be used in the statistical analyses”. In the methods section the Mantel-Haenszel test is mentioned. Importantly, all potential confounders need to be considered in the analysis. Why do the authors not use a multivariate binary logistic regression analysis for primary endpoint analysis?

Reply: We collected patients’ data including body mass index (BMI), presence of diabetes mellitus, smoking status, the use of prophylactic antibiotics, and mode of skin closure. Furthermore, wound protectors are used in all patients. This information has been added in the Methods section (page 16, lines 5-6).

Our study aimed to compare the treatment effects; therefore, the adjusted risk ratio and its 95% confidence interval (CI) will be estimated. In randomized controlled trials with binary outcomes, the risk ratio is the preferred measure of the effect. However, you suggested that multivariate binary logistic regression is the most popular regression model for binary outcomes. Logistic regression analysis yields an odds ratio that approximates the risk ratio when the risk of outcome is low. If the risk of the outcome is low, the difference between the risk ratio and the odds ratio will be negligible, so the adjusted odds ratio estimated using logistic regression can approximate the adjusted risk ratio. However, if the risk of the outcome is high, the adjusted risk ratio should be estimated using the Mantel-Haenszel method or log-binomial regression model. Our study assumes that the risk of outcome is high; hence, the adjusted risk ratio and its 95% CI will be estimated by using the Mantel-Haenszel method.

Comment 7: The authors claim to perform an outcome-assessor blinded trial. However, they state (page 15, 31 “…Diagnosis of SSI, which is reported by nonblinded investigators, will be verified by chart review, and blinded investigators will verify the diagnosis”). This is NOT sufficient masking of the outcome assessors, as only those patients will be assessed by the blinded investigators that have been “reported” by the unblinded investigators. Much rather, ALL patients need to be visited by blinded outcome assessors at fixed postoperative intervals.

Reply: We apologize that our intended meaning was unclear. Non-blinded investigators answer the questionnaire about the wound condition; however, they do not diagnose the presence or absence of SSI. SSIs are diagnosed by investigators who are blinded to the group allocation with reference to the questionnaire. This information has been clarified in the Methods section (pages 13 and 14, lines 16-2).

Comment 8: Please outline in more detail how blinding is maintained. Are operation reports blinded? Is unblinding possible (e.g. in the case of a serious adverse event). Why is data entry not blinded (p19, 41)? This should be implemented. Similarly, data analysts can be blinded. Please specify for each step of the trial who is blinded. In surgical trials the term “single- “or “double-blind” is meaningless and confusing. We recommend:

a. Surgeon: unblinded

b. Patient: blinded

c. Outcome assessor: blinded

d. Data entry: blinded

e. Data analyst: blinded.

Reply: As we described earlier, the non-blinded investigators answer the questionnaire about the wound condition and skin conditions related to adverse events; however, they do not diagnose the presence or absence of SSI and adverse events. SSIs and adverse events are diagnosed by investigators who are blinded to the group allocation with reference to the questionnaire. Blinded investigators perform data entry for the diagnosis of SSI, and the data analyst is blinded. This information has been clarified in the Methods section (pages 13 and 14, lines 16-2).

Comment 9; Please be more specific in defining your outcome measures (erythema, etc.) The primary outcome is SSI according to the CDC definition.

Reply: Erythema is skin redness of the skin that spreads away from the incision site, and it indicates SSI. Localized swelling indicates a bulge limited to the incision site, and tenderness indicates pressured pain beyond normal for the operation. This information has been added to Supplemental Table 1. Furthermore, the presence of skin redness, pruritus, dermatitis, and other symptoms around the region disinfected by an antiseptic during surgery are recognized as intervention-related toxicity. These details have been added to Methods section (page 15, line 13).

Comment 10: Please be very specific whether you want to analysis ALL (i.e. superficial, deep and organ-space) SSIs as primary endpoints or only superficial and deep SSIs. Given the mode of action of a skin disinfectant it sounds reasonable to assume that only infections by skin pathogens will be reduced. This is supported by antimicrobial analyses of SSIs in gastrointestinal surgery. Thus, most likely, only superficial and deep wound infections will be influenced by the intervention. Why are organ-space infections included in the primary endpoint analysis? Do the authors think that organ-space infections like anastomotic leakage, infected pancreatic fistula etc. (all counting as organ-space infections) are influenced by the choice of skin disinfectant?

Reply: As you pointed out, antiseptics would influence only superficial and deep SSIs. However, this is the first study to use olanexidine; therefore, it is more important to establish evidence for all types of SSI than limiting the study to superficial and deep wound infections. Some studies have investigated skin antisepsis in gastrointestinal surgery and included organ SSI as an outcome.[2,3] In our study, we are examining the rate of superficial and deep wound infections as a secondary outcome. This information has been clarified in the Discussion section (page 21, lines 10-14).

Comment 11: Similarly, the protocol needs to clarify the statistical analysis populations. This is not trivial in SSI trials. We recommend performing the primary endpoint analysis in the ITT population rather than using the “the full analysis set (FAS)”. Definition of the FAS by the authors (“…took at least one dose of treatment during the study, did not have any serious violation of the study protocol, and had data collected after treatment commencement”) is vague and not adapted to the current trial. What is meant with “took at least one dose of treatment”? Furthermore, the FAS introduces bias as patients can be excluded at the discretion of the investigators (“had data collected…, did not have a

serious violation of the study protocol…"). We strongly recommend using the ITT population for analysis and the PP and SSI population for sensitivity analysis. Definitions could be as follows:

a. Intention-to-treat population comprises all patients randomized and these patients will be analyzed as randomized. However, a number of patients will not be able to undergo visit at postoperative day 30, either due to death, relaparotomy or lost to follow-up. The authors need to specify for each of these cases how the primary endpoint is imputed in these cases. E.g. is death counted as SSI?

b. Per-protocol population: Patients are analyzed as treated, not as randomized and only those patients are considered that have finished follow-up at day 30.

c. SSI population: all patients randomized that have fulfilled their follow-up at day 30.

Reply: The intention-to-treat (ITT) principle implies that the primary analysis should include all randomized subjects. Compliance with this principle would necessitate complete follow-up of all randomized subjects for study outcomes. In practice, this ideal may be difficult to achieve. The International Conference on Harmonisation E9 guideline on "Statistical Principles for Clinical Trials" uses the term "full analysis set" to describe the analysis set that is as complete as possible and as close as possible to the ITT ideal of including all randomized subjects. The E9 guideline recommends that the full analysis set be used as a conservative strategy in many clinical trials and under many circumstances, and that it provides estimates of treatment effects more likely to mirror those observed in subsequent practice. Consequently, in an analysis according to the ITT principle, the original randomization and number of patients in the treatment groups remain unchanged, the analysis population is as complete as possible, and a potential bias due to exclusion of patients is avoided. Thus, the patient set used for the primary analysis according to the ITT principle is called the "full analysis set."

Additionally, we considered the patients who died or underwent reoperation because of SSI as having SSI. Further, we explained the statistical analysis set in the Methods section as follows (page 18, lines 6-11):

"We will perform the primary analyses using the full analysis set, from which patients who do not undergo surgery or who withdraw consent before assessment of the primary endpoint are excluded. In addition, we will repeat the analyses in the per-protocol set, further excluding patients with major protocol deviations. The safety analysis set will include all patients who were randomly assigned to a study group and received treatment during the study."

Comment 12: The follow-up visits need to be clarified. At what days are patients seen? by whom? and how are assessors blinded?

Reply: Participants will undergo outpatient observation at least once within 30 days. They have to visit the outpatient clinic or an emergency department if they experience any symptoms suggestive of an SSI such as pain or redness. Non-blinded investigators answer the questionnaire about the wound condition; however, they do not diagnose the presence or absence of SSI. SSIs and adverse events are diagnosed by investigators who are blinded to the group allocation with reference to the questionnaire. This information has been clarified in the Methods section (page 14, lines 4-9).

Minor comments:

Comment 13: The CDC SSI definition is well known. There is no need to repeat it in the protocol. Please delete or put it into the supplement.

Reply: According to your suggestion, we included the Centers for Disease Control and Prevention's definition of SSI into the supplemental table.

Comment 14: Exclusion criterion 4: "Receipt of antimicrobial therapy…" please delete "receipt of".

Reply: According to your suggestion, we have deleted "receipt of."

Comment 15: There are contradictory statements concerning the date of initiation (Abstract. June 2018, methods section: May 2018). Please clarify

Reply: According to the reviewer's suggestion, we have changed "May 2018" to "June 2018" in the Methods section (page 12, line 10).

Comment 16: Page 11, 21 please replace "useful" with "more effective".

Reply: According to the reviewer's suggestion, we have replaced "useful" with "more effective" on page 22, line 8.

Reviewer: 2

Comment 1: The study is conducted in four hospitals and includes various gastrointestinal surgeries with different risk level of developing SSIs. No stratification is planned. The methodology used and the data collected are insufficiently developed in the manuscript. Practices may vary from one surgeon and/or anaesthetist to another. Thus, it is impossible to know if the 2 groups will be comparable in terms of risk factors for developing SSI at the end of the study. How imbalances between groups will be taken into account when performing the statistical analyses should be added in the manuscript.

Reply: All surgeries were conducted by Japanese board-certified surgeons, and all surgeons received education from the Department of Surgery, Keio University School of Medicine. Furthermore, since the study was randomized, patients' background characteristics and risk factors are not biased. Furthermore, because the study was blinded, the bias is low.

Comment 2: No information is given concerning the monitoring of the study in order to know if the protocol is respected by all healthcare providers.

Reply: Central monitoring will be conducted with the aim of ensuring that the trials are conducted safely and in accordance with the implementation plan, and that the data collection is correct. It is conducted once a year, with 10% of registration completed in each institution. The number of consents acquired, number of patients registered, number of patients who withdrawal or are lost to follow-up and their reasons, safety, compliance of eligibility criteria and exclusion criteria, accuracy of the allocation procedure, and compliance with various regulations and research plan are all evaluated

by test secretariat. This information has been added to Methods section (pages 16 and 17, lines 17-7).

Comment 3: Little information is given on the 2 antiseptics used and how they will be applied. In addition, the choice of povidone-iodine in aqueous solution as the reference group appears unjustified in 2018 – A large literature has demonstrated the superiority of alcoholic formulations over aqueous formulations of antiseptics.

Reply: The antiseptic should be applied widely in consideration of the drain site and length of the skin incision. We apply agents on the papilla (in cases of esophageal surgery: the neck) with a cranial limit and to the upper thigh with a caudal limit. After waiting 3 minutes to allow the antiseptics to dry, the operation is started. This information has been added in the Methods section (page 11, lines 5-9).

Many studies have indicated the antiseptic superiority of chlorhexidine-alcohol compared to povidone-iodine; however, few randomized trials have reported superiority in clean-contaminated gastrointestinal surgery. Furthermore, since inflammability is associated with chlorhexidine-alcohol, povidone-iodine is recommended and typically used for gastrointestinal surgery in Japan. In addition, chlorhexidine-alcohol with concentrations greater than 1% are not commercially available in Japan, although the concentration greater than 2% is recognized as having a bactericidal effect in international guidelines.

Moreover, considering the influence of ethnic differences including intrinsic and extrinsic ethnic factors,[1] this trial is a meaningful examination of SSI treatment at least in Japan. Therefore, we think that the selection of a control group is reasonable.

As you noted, in the future, we should also consider conducting another trial that compares olanexidine to an alcohol-based antiseptic agent, if superiority of olanexidine compared to povidone-iodine is proven in the present trial. This information has been corrected in the Discussion section (page 21, lines 1-9).

Abstract.

Comment 4: Introduction should better describe the issue being studied and how this study could improve clinical practices.

Reply: According to the reviewer's suggestion, we have addressed the issue in the face of SSI and how our results can improve clinical practices. This information has been added in the Introduction section of the Abstract (page 3, lines 3-11).

Comment 5: What is a Class II surgical wound?

Reply: According to the reviewer's suggestion, we have deleted “class II” and added “clean-contaminated gastrointestinal surgeries” on page 3, line 14.

Comment 6: No information is provided on the approval of the study by an ethics committee.

Reply: According to the reviewer's suggestion, we have added details of the approval process in the Ethics and dissemination section as follows (page 4, line 3): “The protocol was first approved by the Institutional Review Board of Keio University School of Medicine, followed by the institutional review board of each participating site.” This information has also been corrected in the Abstract section (page 4, line 3).

Strengths and limitations.

Comment 7: The second point is neither a strength nor a limitation of the study and should be deleted.

Reply: According to your suggestion, we have deleted the second point of this section.

Comment 8: At least one sentence on the limits of the study should be added.

Reply: We have added the following limitation to this section (page 5, lines 8-9): “This study is limited to a Japanese population, which could introduce an element of selection bias, because olanexidine is only commercially available in Japan.”

Introduction.

Comment 9: Gastrointestinal surgery is a clean-contaminated surgery where the microorganisms involved in ISS come from the skin and digestive tract. Therefore, skin preparation may prevent only a fraction of the SSIs (those due to skin bacteria). This point should be discussed by the authors.

Reply: As you pointed out, antisepsis would influence only superficial and deep SSIs. However, this is the first report to use olanexidine; therefore, it is more important to establish the evidence for all types of SSI than limiting the study to superficial and deep wound infections. Some studies have investigated skin antisepsis in gastrointestinal surgery and included organ SSI as an outcome.[2,3] In our study, we are examining the rate of superficial and deep wound infections as secondary outcomes. This information has been clarified in the Discussion section (page 21, lines 10-14).

Comment 10: Similarly, the superiority of alcoholic formulations over aqueous formulations of antiseptics is not addressed.

Reply: As we described earlier, since inflammability is associated with alcoholic formulations, aqueous povidone-iodine is recommended and typically used for gastrointestinal surgery in Japan. Therefore, we used aqueous iodine as a control group. This information has been added in the Discussion section (page 21, lines 1-9).

Comment 11: WHO guidelines (ref 8) recommend the use of alcoholic chlorhexidine for skin preparation before surgery.

Reply: Because inflammability and inconsistency is associated with chlorhexidine-alcohol, povidone-iodine is recommended and typically used for gastrointestinal surgery in Japan rather than alcoholic chlorhexidine. Chlorhexidine-alcohol with concentrations greater than 1% are not commercially available in Japan. This information has been added in the Discussion section (page 21, lines 1-9).

Comment 12: Chlorhexidine is less effective against MRSA than against MSSA, but it is not true that chlorhexidine is ineffective against MRSA or VRE.

Reply: According to your suggestion, we have deleted this information from the Introduction section.

Comment 13: Is olanexidine available outside Japan?

Reply: To date, olanexidine is only commercially available in Japan.

Comment 14: Olanexidine is bactericidal and not bacteriostatic

Reply: According to the reviewer's suggestion, we have replaced “bacteriostatic” with “bactericidal” (page 7, line 14).

Comment 15: Please specify whether olanexidine is used in alcoholic or aqueous formulation and whether the comparator is aqueous of alcoholic povidone iodine – also indicate the concentration of povidone iodine.

Reply: Both olanexidine and povidone-iodine are used in an aqueous formulation, and 10% povidone iodine is used in this study. This information has been specified in the Methods section (pages 10 and 11, line 17-3).

Methods

Comment 16: Please explain what a class 2 surgical wound is

Reply: Class II surgical wound is a clean-contaminated wound. Operative wounds in which the respiratory, alimentary, genital, or urinary tracts are entered under controlled conditions and without unusual contamination are allocated as class II wounds. This information has been included in the Table 1.

Comment 17: More information is needed on how to apply antiseptics (number of applications, modality, time….). Particularly, do you use olanexidine applicators?

Reply: The antiseptic should be applied widely in consideration of the drain site and length of the skin incision. We apply agents on the papilla (in cases of esophageal surgery: the neck) with a cranial limit and to the upper thigh with a caudal limit. After waiting 3 minutes to allow the antiseptics to dry, the operation is started. One application of olanexidine will be used for surgery; however, if surgeons determine that disinfection is inadequate, an additional application can be applied. This information has been added in the Methods section (page 11, lines 5-11).

Comment 18: In addition, more information is needed on other measures applied to prevent SSI before, during and after surgery, as well as how they will be monitored (shower, hair removal, antibiotic prophylaxis, normothermia....).

Reply: We used other measures to prevent SSI in our protocol as follows:

(1) Administer standard antibiotic prophylaxis before making the surgical incision,

(2) use absorbable sutures for wound closure and recommend the use of antimicrobial-coated sutures,

(3) recommend the use of a wound protector,

(4) recommend wound irrigation,

(5) use any type of immunosuppressive agent,

(6) change or retain the same gloves during the operation, and

(7) change or retain the surgical instruments.

Furthermore, we always maintain a normal body temperature by using warming devices during surgery, and do not perform hair removal before surgery. This information has been added in the Methods section (pages 11 and 12, lines 13-5).

Comment 19: Data collection. All risk factors and prevention methods of SSI should be collected during the perioperative period. These data will be useful to adjust the results in cases of differences between groups.

Reply: We collected patients' data including BMI, diabetes mellitus, smoking status, the use of prophylactic antibiotics, mode of skin closure, and prevention methods of SSI such as standard antibiotic prophylaxis and use of a wound protector. This information has been added in the Methods section (page 16, lines 5-6).

Comment 20: Sample size calculation. The incidence of SSIs with use of aqueous povidone iodine observed in previous studies should be added to support their estimation.

Reply: The SSI rate of 12% was estimated with reference to our previous report. However, this Japanese report was not published. This information has been added in the Methods section (page 17, lines 10-11).

Comment 21: Statistical analysis. "one dose of treatment" is not appropriate

Reply: According to the reviewer's suggestion, we have deleted "one dose of."

Comment 22: Fig 1 is a table

Reply: According to the reviewer's suggestion, we have included Figure 1 as a table.

Comment 23: A figure with the flow chart of the study is missing

Reply: The schedule for the trial visits and data collection is summarized in the Methods section (page 14, lines 4-9) and Table 2.

VERSION 2 – REVIEW

REVIEWER	Markus W. Büchler Department of Surgery
REVIEW RETURNED	29-Jan-2019

GENERAL COMMENTS	My comments were addressed fully.

REVIEWER	Olivier MIMOZ University Hospital of Poitiers, France
REVIEW RETURNED	17-Jan-2019

GENERAL COMMENTS	I would like to thank the authors for revising their manuscript based on my comments. Although much has been done, I think two points still deserve the authors' attention. 1. The authors should add the duration of application of each antiseptic and the method of application (compresses on pliers or ready-to-use applicators). 2. With regard to other methods of preventing SSIs, it is necessary to specify: 2.1 Wound protectors are with or without anti-infective agents and if so which ones, 2.2 What type of solution is used to irrigate the wound, 2.3 Modify the following mistakes: "use any type of immunosuppressive agent", "change or keep the same gloves during the operation", "change or keep surgical instruments".

VERSION 2 – AUTHOR RESPONSE

Reviewer: 2

Comment 2: The authors should add the duration of application of each antiseptic and the method of application (compresses on pliers or ready-to-use applicators).

Reply: The duration of application of both antiseptics is at least 1 minute.

Olanexidine is administered by ready-to-use applicators, and povidone-iodine is administered by a brush or by compression using pliers (Page 11).

Comment 3: With regard to other methods of preventing SSIs, it is necessary to specify:

1 Wound protectors are with or without anti-infective agents and if so which ones,

Reply: Although we recommend the use of a wound protector, whether it is actually used is decided by the operator. The types used are the Alexis® wound protector (Medical Leaders Co. Ltd, Japan) or the lap protector (HAKKO Co. Ltd, Japan), which are used without anti-infective agents (Page 12).

2 What type of solution is used to irrigate the wound,

Reply: Incisional wound irrigation is performed with sterile normal saline (Page 12).

3 Modify the following mistakes: "use any type of immunosuppressive agent", "change or keep the same gloves during the operation", "change or keep surgical instruments".

Reply: According to the reviewer's suggestion, we have revised these mistakes as follows:

(5) Not restricting the type of immunosuppressive agent that can be used

(6) Changing or maintaining the same gloves during the operation

(7) Changing or maintaining the surgical instruments (Page 12)