Appendix 2 Modified Strobe AMS checklists

2 examples provided.

Study (1)	**Item #**	**Recommendation**	**Reported Yes**	**Page #**	**Relevant Text from manuscript**
Title and Abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	✓	1	Antimicrobial Activities of Methanol, Ethanol and Supercritical CO2 Extracts of Philippine Piper betle L. on Clinical Isolates of Gram Positive and Gram Negative Bacteria with Transferable Multiple Drug Resistance
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	✓	1	Abstract describes background, assay methods, population MDR bacterial isolate, results; This study revealed the bactericidal activities of all the P. betle leaf crude extracts on methicillinresistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus (VRE), extended spectrum β-lactamase-producing Enterobacteriaceae, carbapenem-resistant Enterobacteriaceae, and metallo-β-lactamase-producing Pseudomonas aeruginosa and Acinetobacter baumannii, with minimum bactericidal concentrations that ranged from 19μg/ml to 1250 μg/ml. and conclusions: P. betle as an alternative source of anti-infectives against multiple drug resistant bacteria.
Introduction					
Background Rationale	2	Explain the scientific background and rationale for the investigation being reported	✓	2	...all these aforementioned studies did not determine the antibacterial activities of the P. betle extracts on the more novel multiple drug resistant (MDR) bacterial strains identified by the Infectious Disease Society of America (IDSA) as especially difficult to treat, and which the present study specifically addressed
Objectives	3	State specific objectives, including any pre-specified hypotheses	✓	3	... present study ...aimed to determine and compare the antimicrobial potencies of the methanol, ethanol and supercritical CO2 extracts of the Philippine P. betle on a larger number of MDR bacteria isolated from recent clinical cases in tertiary hospitals in the Philippines.
Methods					

Study Design	4	Present key elements of study design early in the paper	✓	1	This study determined the antimicrobial activities of its ethanol, methanol, and supercritical CO2 extracts on clinical isolates of multiple drug resistant bacteria which have been identified by the Infectious Disease Society of America as among the currently more challenging strains in clinical management. Assay methods included the standard disc diffusion method and the broth microdilution method for the determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentrations (MBC) of the extracts for the test microorganisms
Settings	5	Describe the setting	✓	1	Institute of Biology, College of Science, University of the Philippines, Diliman, Quezon City, 1101, Philippines
Subjects	6	Identification of specific bacterial strain. Identification of specimen as multidrug resistant (MDR) bacteria	✓ ✓	4	The MDR bacterial strains used in this study, together with their resistance phenotypes are listed in Table 1. All isolates were retrieved from the Microbial BioBanks of the Makati Medical Center and Ospital ng Makati, which maintain microbial isolates collected from patients' clinical specimens. Both are Level III training hospitals located in Makati City, Philippines. The patients from which the bacteria were isolated were anonymized, on the basis for which informed consent from the patients was not required by the respective Institutional Review Boards. All isolates were identified by automated biochemical tests using Vitek1MS (bioMérieux, Marcy l'Etoile, France) GP colorimetric identification card. The susceptibility patterns were determined using Vitek1MS AST (bioMérieux, Marcy l'Etoile, France) following the MIC interpretative standards of the Clinical Laboratory Standard Institute M100-S24 [25].
Intervention	7	Reporting of Plant derived antimicrobial (PDAm) species name, part used. Identification of extraction solvent used ethanol / methanol and plant preparation and solvent and strength	✓ ✓	4	The extraction was done on the powdered dried leaves of P. betle following the methods of Basri and Fan [23] with minor modifications. The powdered plant materials in the amount of 150 g were soaked in 500 ml of absolute ethanol and absolute methanol separately for seven days with occasional stirring, and then filtered using Whatman filter paper no.1 (Whatman Ltd., England). The filtrate was concentrated under reduced pressure using a rotary evaporator at 50°C. The crude extract was collected and allowed to completely dry at room temperature

Antimicrobial Susceptibility Test (AST)	8	(a) Reports Clinical & Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines are met. (b) Describes AST method used (diffusion or dilution) including inoculum preparation method, inoculum size, growth medium, atmosphere, temperature, duration of incubation and end-point determination.	✓ ✓	5 4-5	The susceptibility patterns were determined using Vitek1MS AST (bioMérieux, Marcy l'Etoile, France) following the MIC interpretative standards of the Clinical Laboratory Standard Institute M100-S24 Disk Diffusion Method. The test bacteria were grown on sheep blood agar plates for 16– 18 hr. at 35± 2°C. Well-isolated colonies were suspended in sterile 0.9% saline solution and the turbidity was adjusted against 0.5 McFarland standard to comprise approximately 1.5 x 108 CFU/ml. The inoculum was swabbed on the surface of Mueller-Hinton Agar plate (Remel Inc. USA) using sterile cotton swab. Sterile 6-mm blank disks (Becton Dickinson and Company, USA) were loaded with 25 ul of diluted plant extract stock solution giving a dry weight concentration of 2.5 mg/disc. Representative antibiotic disks per bacterial strain and disks with 0.2% DMSO served as positive and negative controls, respectively. The plates were incubated at 35 ± 2°C for 16 to 24 hours. The diameters of the zones of inhibition produced by the plant extracts on test on the test isolates measured in mm. Broth Microdilution Method. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the plant extracts were determined using the broth microdilution method based on Clinical Laboratory Standard Institute M07-A8 [26]. Two-fold serial dilution of each plant extract with starting concentration of 100 mg/ml was prepared using cation-adjusted Mueller-Hinton broth or MHB (Becton Dickinson and Company, USA) as diluent resulting in concentrations of 2.44 µg/ml to 10,000 µg/ml. Each set-up was carried out in triplicate in sterile 96-well microplates. Controls consisted of culture control (no plant extract), negative control (plant extract and MHB only) and reference drug controls
Data sources/ measurement	9	Describes how antimicrobial activity was measured. MIC/IC50/ZOI	✓	8	Minimum inhibitory concentration (MIC) in mg/mL
Statistical methods	10	Describe all statistical methods used.	✓	5	All the experiments were carried out in triplicate and mreresetned by the mean

Results					
Interventions	12	Provides results of AST (MIC/IC50/ZOI)	✓		The diameters of the zones of inhibition produced by the plant extracts on test on the test isolates measured in mm. Broth Microdilution Method. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC)
Descriptive Data	13	Provides description of these results in terms of qualitative/quantitative.	✓	5	ZOI mm MIC μg/ml
Main results	14	Results provide qualitative data ZOI in mm or alternatively quantitative data MICS, MBS and ZOI described as susceptible, intermediate and resistant	✓	7 9	Table 2. Diameters of zones of inhibition (mm)* of Piper betle extracts against multidrug-resistant bacteria. Table 3. Minimum inhibitory concentrations (μg/ml) of Piper betle extracts for multidrug-resistant bacteria.
Discussion					
Key Results	15	Summarise key results with reference to study objectives	✓	10-11	Results suggest that the antibacterial compounds in the crude extracts were recalcitrant to the different resistance mechanisms of these MDR isolates. This is most worthy to note since it responds to the pressing need for new antimicrobial compounds that are not targets of the drug-inactivating enzymes ESβL and carbapenamases (including the MβLs), and can still effectively function as antibacterials in the presence of the modified penicillin binding protein (PBP2A) in MRSA strains, and peptidoglycan receptors with reduced vancomycin affinity present in the VRE strains. The crude extracts did not only inhibit the growth, but were bactericidal for all the test organisms with MBCs in the range of 19 μg/ml to 1250 μg/ml. The different extracts proved to be more potent against the Gram-positive, especially for the VRE strains, than for the Gram-negative MDR bacteria. Studies have reported the role of the outer membrane in Gram-negative bacterial cell wall as a permeability barrier in conferring antibiotic resistance [29, 30, 31, 32]. Generally, the ethanol extract proved to be more potent than the methanol and supercritical CO2 extracts

Limitations	16	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	✓	14	spur further studies on the identification and purification of the active antibacterial compounds that would lead to eventual clinical application
Interpretations	17	Give a cautious overall interpretation of results and other relevant evidence. Conclusions match results	✓	11-12	Data of the study thus firmly show the promising potential use of P. betle compounds against both Gram-positive and Gram-negative MDR bacteria. This should spur further studies on the identification and purification of the active antibacterial compounds that would lead to eventual clinical application.
Other information					
Funding		Give the source of funding and the role of the funders for the present study	✓	1	This work was supported by Philippine Council for Health Research and Development of the Department of Science and Technology, Philippines, to DLV. http://www.pchrd.dost.gov.ph/. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Sources:
STROBE-AMS: recommendations to optimise reporting of epidemiological studies on antimicrobial resistance and informing improvement in antimicrobial stewardship (2). STROBE Statement—checklist (3)

1. Valle DL, Jr., Cabrera EC, Puzon JJ, Rivera WL. Antimicrobial Activities of Methanol, Ethanol and Supercritical CO2 Extracts of Philippine Piper betle L. on Clinical Isolates of Gram Positive and Gram Negative Bacteria with Transferable Multiple Drug Resistance. PLoS One. 2016;11(1):e0146349.
2. Tacconelli E, Cataldo MA, Paul M, Leibovici L, Kluytmans J, Schröder W, et al. STROBE-AMS: recommendations to optimise reporting of epidemiological studies on antimicrobial resistance and informing improvement in antimicrobial stewardship. BMJ Open. 2016;6(2):e010134.
3. (STROBE) TStRoOsiE. STROBE checklists 2007 [updated Nov 2007 Available from: https://www.strobe-statement.org/index.php?id=available-checklists.

Study (1)	**Item #**	**Recommendation**	**Reported Yes**	**Page #**	**Relevant Text from manuscript**
Title and Abstract	1	(a) Indicate the study's design with a	✓	1	In vitro antibacterial activity of Quercus infectoria gall extracts against

		commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found	 ✓		multidrug resistant bacteria Abstract describes background, methods including MDR bacterial strains and assays, results and conclusions
Introduction					
Background Rationale	2	Explain the scientific background and rationale for the investigation being reported	✓	2	it would be beneficial to look for plantderived antimicrobial compounds which are useful as an alternative strategy in the treatment of infections related to antimicrobial resistance bacteria
Objectives	3	State specific objectives, including any pre-specified hypotheses	✓	2	this study was conducted to evaluate the antibacterial activity of aqueous and ethanol gall extracts of Q. infectoria against few selected strains of MDR bacterial isolates
Methods					
Study Design	4	Present key elements of study design early in the paper	✓	2	The current preliminary study was designed to obtain data on the in vitro anti-bacterial activity of Q. infectoria galls extracts towards selected MDR bacteria strains
Settings	5	Describe the setting	✓	2	Medical Microbiology and Parasitology Laboratory, School of Medical Sciences, Universiti Sains Malaysia (USM)
Subjects	6	Identification of specific bacterial strain. Identification of specimen as multidrug resistant (MDR) bacteria	✓ ✓	2-3	Staphylococcus aureus (MRSA), methicillin resistant coagulase negative Staphylococcus (MRCoNS), multidrug resistant Acinetobacter sp. (MDR Acinetobacter sp.), extended spectrum beta lactamase Escherichia coli (ESBL E. coli) and extended spectrum beta lactamase Klebsiella pneumoniae (ESBL K. pneumoniae). Staphylococcus aureus ATCC 25923 and MRSA ATCC 43300 were used as reference strains.

Intervention	7	Reporting of Plant derived antimicrobial (PDAm) species name, part used. Identification of extraction solvent used ethanol / methanol and plant preparation and solvent and strength	✓ ✓	2	The galls of Q. infectoria used in this study were obtained from the local market in Kota. Successive solvent extraction was performed for S. striata. Plants were washed, air dried for 7-8 days, and ground into powder before they were placed into the flask of the Soxhlet apparatus for extraction using 70% ethanol with increasing order of polarity to extract the phytoconstituents separately at 20ºC for 3-4 h Ethanol HPLC grade obtained from SigmaAldrich, Germany).
Antimicrobial Susceptibility Test (AST)	8	(a) Reports Clinical & Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines are met. (b) Describes AST method used (diffusion or dilution) including inoculum preparation method, inoculum size, growth medium, atmosphere, temperature, duration of incubation and end-point determination.	✓ ✓	2 3	Each isolate was identified based on the standard biochemical tests and the resistance to different antimicrobial agents was determined using the disc diffusion method standardized by the CLSI recommendations Sterile filter paper discs (Whatman No. 1, 6 mm) were impregnated with 20 µl of each of the extracts (50 mg/ml) to give the final concentration of 1 mg/disc. The discs were left to dry in the fume hood overnight. Mueller Hinton agar was used as the media for the test microorganisms. The bacterial inoculum was spread evenly onto the surface of the Mueller Hinton agar using a sterile cotton swab followed by placing the impregnated discs onto the inoculated agar surface. Three replicates of each extract were assayed and the mean values were then referred. Discs containing sterile distilled water served as negative control. Vancomycin (30 µg) and imipenem (10 µg) discs were used as positive control for the Gram positive and ESBL Gram negative bacteria respectively. Colistin (10 µg) disc was used as positive control for MDR Acinetobacter sp. All plates were incubated at 37ºC for 24 hours. The antibacterial activity was interpreted based on the diameter of clearing zone measured to the nearest millimeter (mm) surrounding the disc indicating growth inhibition. The minimum inhibitory concentration (MIC) value of each extract was determined for bacterial strains which exhibited inhibitory zones. The assay

					was performed using twofold serial microdilution in the 96-well microtiter plate as described previously by CLSI (2012). Briefly, 100 µl of Mueller-Hinton broth was pipetted into test and control wells (growth and sterility controls). Subsequently, twofold serial dilution of extracts was performed in the test wells giving rise to concentrations ranging from 5.00 mg/ml to 0.01 mg/ml. Then, 5 µl of the diluted bacterial suspensions (final inoculum of 105 bacteria per ml) were added to the wells mixed thoroughly. The extracts in broth were used as negative control to ensure medium sterility while the bacterial suspensions in broth served as positive control to control the adequacy of the broth for bacterial growth. Each extract was assayed in triplicates. After an overnight incubation at 37ºC, the MIC values were taken as the lowest concentration of the extracts in the wells that showed no turbidity. Subsequently, the minimum bactericidal concentration (MBC) value was determined by sub-culturing the wells which showed no turbidity onto nutrient agar plate. The lowest concentration of extract showing no visible growth on the agar plate after an overnight incubation at 37ºC was considered as MBC value.
Data sources/ measurement	9	Describes how antimicrobial activity was measured. MIC/ZOI	✓	4-5	Inhibition zone diameter (mm ± SEM)†). MIC (mg/ml) MBC (mg/ml) MBC/MIC ratio
Statistical methods	10	Describe all statistical methods used.	✓	3	Mean value of three determinations, each from different plates. Statistical analysis GraphPad Prism 6 software was used for data entry and statistical analysis. Independent T-test was used for statistical comparison of the mean values for inhibition zone diameter obtained from aqueous and ethanol extracts.
Results					
Interventions	12	Provides results of AST (MIC/ZOI) or other	✓		ZOI MIC Inhibition zone diameter (mm ± SEM) MIC (mg/ml)
Descriptive Data	13	Provides description of these results in terms of qualitative/quantitative.	✓		MIC, MBCs and ZOIs quantitative

Main results	14	Results provide qualitative data ZOI in mm or alternatively quantitative data MICS, MBS and ZOI described as susceptible, intermediate and resistant	✓	4-5	Table 1. Antibacterial activity of Q. infectoria gall extracts (1mg/disc) against MDR bacteria by disc diffusion test Table 2. Determination of the MIC values of Q. infectoria galls extracts against MDR bacteria Table 3. Determination of the MBC values of Q. infectoria galls extracts against MDR bacteria Table 4. Summary of MIC and MBC values of Q. infectoria galls extracts against MDR bacteria
Discussion					
Key Results	15	Summarise key results with reference to study objectives	✓	6	In this study, both aqueous and ethanolic gall extracts demonstrated antibacterial activity against MDR bacteria based on disc diffusion test results except for ESBL organisms. The screening test of the extracts against MRSA showed that aqueous extract produced significantly larger inhibition zone as compared to ethanolic extract. Strong antibacterial activities of both extracts were observed not only against MRSA but also MRCoNS and relatively weak inhibitory effects against MDR Acinetobacter sp
Limitations	16	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	✓	7	Testing their effectiveness and toxicity in vivo are warranted in the near future.
Interpretations	17	Give a cautious overall interpretation of results and other relevant evidence. Conclusions match results	✓	3	Both extracts exhibited inhibitory effects against each MDR bacterial species tested except for ESBL enterobacteriaceae (E. coli and K. pneumoniae).
Other information					

Funding		Give the source of funding and the role of the funders for the present study	✓	7	Acknowledgements. This work was supported by the USM short term grant 304/PPSK/ 61312061. We gratefully acknowledge the Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia for providing the stock culture isolates.

Sources:
STROBE-AMS: recommendations to optimise reporting of epidemiological studies on antimicrobial resistance and informing improvement in antimicrobial stewardship (2). STROBE Statement—checklist (3)

1. Wan Nor Amilah WA, Masrah M, Hasmah A, Noor Izani NJ. In vitro antibacterial activity of Quercus infectoria gall extracts against multidrug resistant bacteria. Trop Biomed. 2014;31(4):680-8.
2. Tacconelli E, Cataldo MA, Paul M, Leibovici L, Kluytmans J, Schröder W, et al. STROBE-AMS: recommendations to optimise reporting of epidemiological studies on antimicrobial resistance and informing improvement in antimicrobial stewardship. BMJ Open. 2016;6(2):e010134.
3. (STROBE) TStRoOsiE. STROBE checklists 2007 [updated Nov 2007 Available from: https://www.strobe-statement.org/index.php?id=available-checklists.